Food Research International 72 (2015) 215–222

Contents lists available at ScienceDirect

Food Research International

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

Influence of processing conditions on reducing γ-aminobutyric acid content during fortified milk production

Yaosong Wang [a], Mingying Liu [a], Liming Zhao [a,b,*], Yongjun Qiu [a], Yingping Zhuang [a]

[a] State Key Laboratory of Bioreactor Engineering, R&D Center of Separation and Extraction Technology in Fermentation Industry, East China University of Science and Technology, Shanghai 200237, China

[b] State Key Laboratory of Dairy Biotechnology, Technology Center of Bright Dairy & Food Co., Ltd., Shanghai 200436, China

ARTICLE INFO

Article history:
Received 9 January 2015
Received in revised form 29 March 2015
Accepted 2 April 2015
Available online 11 April 2015

Keywords:
γ-Aminobutyric acid
Milk
Processing conditions

ABSTRACT

The work studied the effects of processing conditions on the γ-aminobutyric acid (GABA) loss during fortified milk production. Bovine milk or their proteins/lactose fractions (0.66% whey protein and 2.6% casein or 4.9% lactose, w/v) containing 0.05–1.0% added γ-aminobutyric acid (w/w, based on bulk milk or these fractions) were subjected to a simulated milk technological process as following the sequential preheating (25–60 °C), homogenization (0–20 MPa), and pasteurization (62 °C/30 min, 72 °C/15 s, 95 °C/5 min, and 138 °C/2 s) or their unit processes to treat GABA. The resulting samples were characterized through GABA and lactose concentrations under various processing conditions. The amine and carboxyl groups and the structural characteristics of the resulting protein (lactose) were also examined through their concentrations (for lactose) and mass/spectral analyses, respectively. The results showed that the increase in temperature significantly promoted a reduction in GABA content. Whey protein fractions than caseins were primarily responsible for inducing GABA, whereas lactose had no remarkable effect on it. The rationale for GABA reduction is potential reactions with milk proteins/lactose, which preliminarily confirmed by the measurement of protein modification and lactose mass spectrometry.

1. Introduction

γ-Aminobutyric acid (GABA) has received growing interest due to its health benefits and novel physiological functions. In particular, it has anti-hypertensive (Hayakawa, Kimura, & Kamata, 2002; Liu et al., 2011; Yamakoshi et al., 2007) and natriuretic effects (Yamakoshi et al., 2007), ameliorates serum cholesterol level (Roohinejad et al., 2009, 2010), and has a protective effect against renal damage (Kim, Yokozawa, Nakagawa, & Sasaki, 2004). Moreover, it has sedative (Franco et al., 2012; Lee et al., 2010), hypolipidemic (Rajasekaran & Kalaivani, 2011), and diuretic effects (Wong, Bottiglieri, & Snead, 2003), as well as anti-diabetic activity (Rajasekaran, Kalaivani, & Sabitha, 2009), and regulates sleep (Cho et al., 2012). These findings suggest that GABA has many biological effects in humans.

Many approaches have been implemented to enrich food with GABA for nutritive value enhancement. Typically, various types of microbes are induced to ferment foodstuffs and transfer glutamic acid to GABA (Di Cagno et al., 2010; Erlander, Tillakaratne, Feldblum, Patel, & Tobin, 1991; Lee et al., 2010; Pyo & Song, 2009; Stromeck, Hu, Chen, & Michael, 2011; Tung, Lee, Liu, & Pan, 2011). Another approach is seed germination (Guo, Yang, Chen, Song, & Gu, 2012; Iimure et al., 2009; Komatsuzaki et al., 2007; Yang, Chen, & Gu, 2011). GABA production procedures must be optimized to target the increase in GABA content in food (Bai et al., 2009; Chung, Jang, Yon, & Lim, 2009; Guo, Chen, Song, & Gu, 2011; Li, Bai, Jin, Wen, & Gu, 2010; Watcharatparpaiboon, Laohakunjit, & Kerdchoechuen, 2010), but these techniques are complicated and require extra economic investment which leads to an increase in processing costs.

One of the simple ways to enrich foods with GABA is direct addition. Simplification of the technical food processing procedure reduces the food manufacturing costs. Fortified milk products could be manufactured to improve nutrition (Nejati et al., 2013; Wu, Tsai, Hwang, & Chiu, 2012). Actually, many studies have developed GABA-enriched foods, such as yogurt (Pyo & Song, 2009), cheese (Nomura, Kimoto, Someya, Furukawa, & Suzuki, 1998; Wang, Cui, Chen, & Zhang, 2011; Wang, Dong, Chen, Cui, & Zhang, 2010), and milk-based beverages (Servili et al., 2011). However, no GABA-fortified liquid milk is commercially available. This type of food product has great potential commercial value in the market.

Though the former successfully fortified dairy products with GABA, the technical process for this food production is quite different from the preparation of liquid milk. The later process requires relatively higher temperature in the treatments of preheating and pasteurization,

* Corresponding author at: State Key Laboratory of Bioreactor Engineering, R&D Center of Separation and Extraction Technology in Fermentation Industry, East China University of Science and Technology, Shanghai 200237, China. Tel./fax: +86 21 64250829.
E-mail address: zhaoliming@ecust.edu.cn (L. Zhao).

http://dx.doi.org/10.1016/j.foodres.2015.04.004
0963-9969/

and also involves homogenization, whereas these process factors could give birth to the unexpected reactions between milk factions (e.g.: whey proteins/casein and lactose) and added GABA and consequently affect milk processing properties and food safety. Till now, relatively little information is available on the GABA fortified liquid milk. Therefore, the objective of this study was to preliminarily elucidate the effects of processing conditions during fortified milk production on the quantitative changes in added GABA. To probe these reactions and critical processing factors, it is necessary to determine the appropriate amount of GABA to fortify milk. Preheating, homogenization, and pasteurization were simulated to probe possible GABA physiochemical reactions during milk production.

2. Materials and methods

2.1. Materials and chemicals

Whey protein isolate (90% protein) was donated by Hilmar Ingredients (Hilmar, CA, USA), and sodium caseinate (90% protein) was purchased from Murray Goulburn Cooperative Co. Ltd. (Murray Goulburn, VIC, Australia). GABA (99% purity) was purchased from Shanghai Bangcheng Chemical Co., Ltd. (Shanghai, China). Fresh milk samples were a gift from Bright Dairy & Food Co., Ltd. and stored at 4 °C until used within 12 h. Because it has been standardized, the whey proteins, casein, and lactose fractions were 0.6% (w/v), 2.6% (w/v), and 4.9% (w/v), respectively. All other chemicals used were of analytical grade or higher and were purchased from Lingfeng Chemicals (Shanghai, China).

2.2. Preparation of GABA-fortified milk

To simulate the practical processing conditions, the main milk fractions such as 0.6% (w/v) whey protein, 2.6% (w/v) sodium caseinate, and 4.9% (w/v) lactose were prepared by stirring dry their powders in distilled water until completely solubilized, and then were added with 0.05–1.0% (w/w, based on the fraction solution) GABA, respectively. For the milk samples, GABA was added to aqueous milk solutions at up to 0.5% (w/w, based on the milk solution) and stirred completely dissolved. All the above samples were subjected to the separate or sequentially combined treatment of 25–60 °C preheating in a water-bath for 2 min, 7–20 MPa homogenization (GYB, 40-10S, Shanghai), and pasteurization treatments (included 62 °C for 30 min, 72 °C for 15 s, 95 °C for 5 min, and 138 °C for 2 s) in an oil-bath. The samples were stored at 4 °C until further analysis.

2.3. GABA determination

GABA concentrations in the samples were determined according to the methods of Herbert, Barros, Ratola, and Alives (2000) and Wu, Lee, and Pan (2009). A 250 μL aliquot of sample was added to 750 μL trichloroacetic acid (3%, w/v), thoroughly mixed, and stored overnight at 4 °C to remove protein. Next, the samples were centrifuged at 15,000 ×*g* for 15 min, and the supernatant was diluted 100-fold with 0.1 M HCl and added to phenyl isothiocyanate and triethylamine (v/v/v, 2:1:1). After vortexing (Scientific Industries Vortex-Genie 2, USA), the samples were stored at 40 °C for 1 h, 1 mL hexane was added, and the mixture stood for an additional 10 min. The treated samples were membrane filtered (0.45 μm thickness), and the permeate

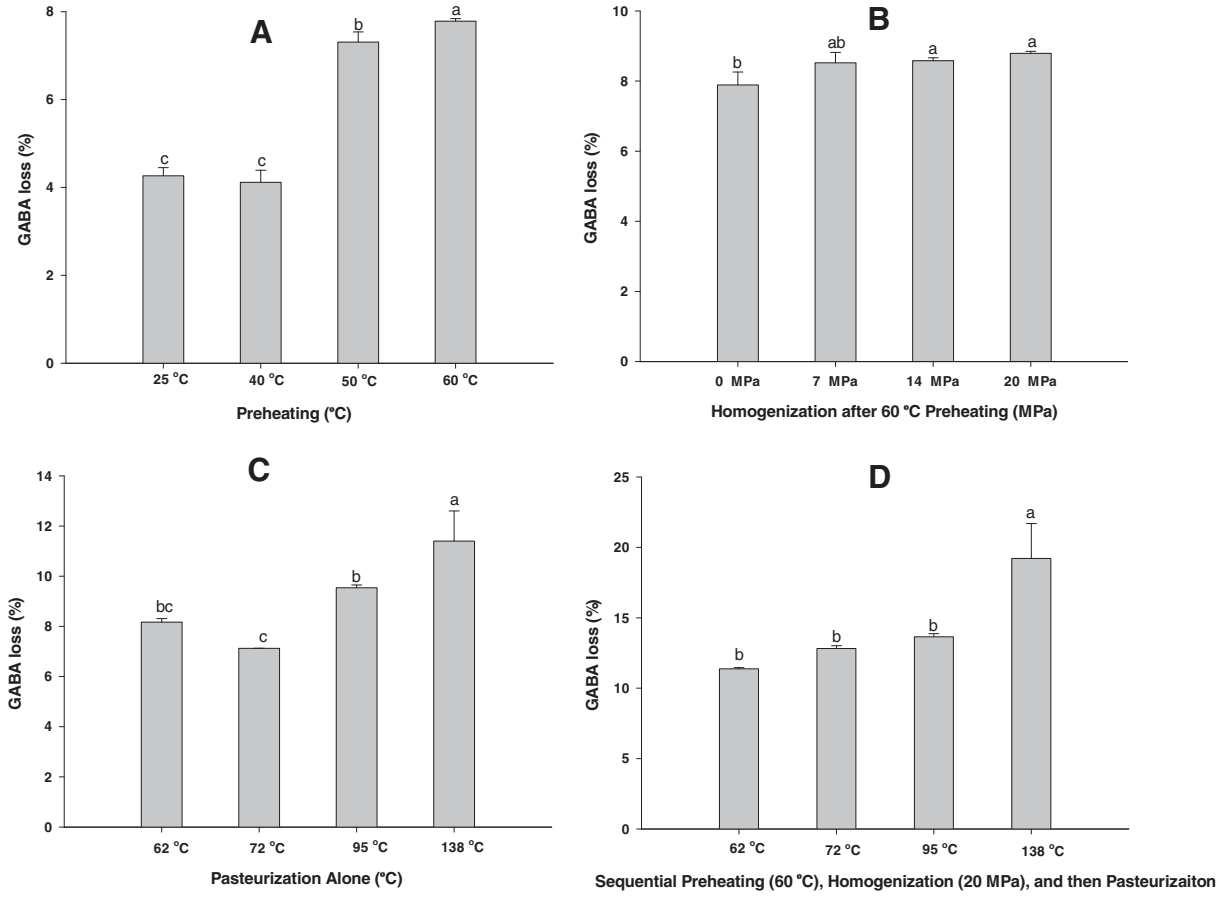

Fig. 1. The unit processes affected γ-aminobutyric acid (GABA) content during milk preparation. A: Preheating at 25–60 °C; B: 0–20 MPa homogenization pressure after 60 °C preheating; C: the different pasteurization conditions were 62 °C for 30 min, 72 °C for 15 s, 95 °C for 5 min, and 138 °C for 2 s; D: sequential 60 °C preheating, 20 MPa homogenization pressure, and pasteurization at different temperatures.