Food Microbiology 26 (2009) 559–564

Contents lists available at ScienceDirect

Food Microbiology

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

Fermented goats' milk produced with selected multiple starters as a potentially functional food

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ARTICLE INFO

Article history:
Received 4 September 2008
Received in revised form
13 January 2009
Accepted 21 March 2009
Available online 27 March 2009

Keywords:
Fermented goats' milk
GABA
ACE-inhibitory activity
Functional

ABSTRACT

A screening among five lactic acid bacteria, used alone or in combination, led to select a mixed starter (*Streptococcus thermophilus* CR12, *Lactobacillus casei* LC01, *Lactobacillus helveticus* PR4, *Lactobacillus plantarum* 1288) capable to produce a fermented goats' milk containing γ-aminobutyric acid (GABA) and angiotensin-I converting enzyme (ACE)-inhibitory peptides. The fermented milk was characterized by cell counts of lactic acid bacteria not lower than 7.0 log cfu g^{-1}, even after 45 days of storage at 4 °C. Fermentation of goats' milk resulted in the production of *ca.* 28 mg kg^{-1} of GABA. Furthermore the fermented goats' milk had an *in vitro* ACE-inhibitory activity of *ca.* 73%. Prolonged cold storage did not significantly affect both the concentration of GABA and the ACE-inhibitory activity. Moreover, the taurine content did not significantly vary during both fermentation and the entire storage period.

1. Introduction

During the last decade, interest of industries and consumers for functional foods has been exponentially increasing. According to the Consensus Document issued by the European Concerted Action on Science of Functional Foods, a food may be referred to as "functional", if it has been unequivocally proven that it positively influences one or more biological functions in the human body, improving the state of health and wellness, and reducing the risk to develop a disease (Diplock et al., 1999). The use of milk with particular nutritional properties (e.g., goats', mares' and donkeys' milk), alone or in combination with bacterial strains having probiotic properties and/or producing physiologically active metabolites, represents one of the technology options for manufacturing new dairy functional beverages (Gomes et al., 1998).

Goats' milk products, especially cheeses and yogurts, are very popular in the Mediterranean peninsula, Middle East, Southern Russia and Indian subcontinent (Tamime and Robinson, 1999). In the European Union, the value of production of goats' milk in 2005 has been estimated 1371 million of euros (http://faostat.fao.org/site/613/DesktopDefault.aspx?PageID=613#ancor).

Goats' milk has been described as having higher digestibility, due to reduced dimensions of casein micelles and fat globules and higher proportion of short to medium fatty acids (Park et al., 2007), and lower allergenic properties than cows' milk (Spuergin et al., 1997). Besides, certain therapeutic properties in human nutrition, such as a better utilization of fat and mineral salts in individuals suffering from malabsorption syndrome, are attributed to goats' milk (Alferez et al., 2001). Goats' milk contains also free taurine, one of the final metabolic products of sulphur-containing amino acids (Park et al., 2007), which may have several biological functions: modulator of growth (Naismith et al., 1987) and of neuronal activity (Haas and Hosli, 1973; Huxtable, 1989; Hussy et al., 1997; Jiang et al., 2004); conjugation of bile salts (Heuman and Bajaj, 1994); regulation of osteoblast metabolism (Koide et al., 1999); protection of cells against various types of injury and prevention of cardiovascular damage (Warskulat et al., 2007); treatment of fatty liver of children (Obinata et al., 1996).

The functional value of goats' milk may be further exploited through fermentation by selected microorganisms possessing specific features. A mixed starter comprising *Lactobacillus acidophilus*, *Bifidobacterium lactis* and *Streptococcus thermophilus* has been successfully used for fermentation of goats' milk (Cutic et al., 2004), and a high viability of probiotic strains in a fermented goats' milk stored at 4 °C for 10 days has been reported (Kongo et al., 2006). Furthermore, the synthesis of folate from some lactic acid bacteria has been shown to occur during fermentation of goats' milk (Sanna et al., 2005), and the

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doi:10.1016/j.fm.2009.03.008

anti-atherogenic effects of a goats' milk fermented with the probiotic *Lactobacillus fermentum* ME-3 have been reported in 16 healthy subjects (Kullisaar et al., 2003).

Milk proteins are currently the main source of a range of biologically active peptides. Many bioactivities are encrypted within the primary structure of milk proteins, requiring proteolysis for their generation from precursors. Proteolysis may produce these biogenic peptides during food processing as well as during gastro-intestinal transit. Enzymes from different sources, including microbial enzymes, generate bioactive peptides during milk fermentation and cheese ripening, thereby enriching the dairy products (Gobbetti et al., 2002). The capacity of several lactic acid bacteria to synthesize angiotensin-I converting enzyme (ACE)-inhibitory peptides in dairy products is well known (Gobbetti et al., 2004; Muguerza et al., 2006; Donkor et al., 2007). The type of lactic acid bacterium used as starter is one of the main factors that influences the synthesis of bioactive peptides in dairy products (Gobbetti et al., 2002). Indeed, it is well known that lactic acid bacteria differ as for proteolytic activity. *In vivo* studies showed that ACE-inhibitory peptides are moderately hypotensive and foods containing them may be considered as co-adjuvant in the treatment of mild hypertension (Seppo et al., 2003). Moreover, it has been shown that lactic acid bacteria have the capacity to synthesize γ-aminobutyric acid (GABA) from L-glutamate through glutamate decarboxylase activity. During milk fermentation a high level of L-glutamate may be theoretically liberated since native caseins contain a high proportion of this amino acid. GABA, a non-protein amino acid, possesses well known physiological functions such as neurotransmission, induction of hypotension, and diuretic and tranquilizers effects (Jakobs et al., 1993; Guin Ting Wong et al., 2003).

This paper reports on investigations aimed at developing and characterizing a fermented goats' milk enriched of GABA and ACE-inhibitory peptides through the use of selected multiple starter cultures.

2. Materials and methods

2.1. Raw milk, microorganisms and ingredients

Milk samples were collected when goats (Ionica breed) were 9–11 weeks in lactation and analysed for chemical composition. Protein content was determined by the Kjeldahl method (AOAC, 2003a) using a VELP DK6 heating digester and a VELP UDK 130 distillation system (VELP Scientifica, Usmate Milan, Italy). Fat was determined by the Gerber method, according to Stelios and Emmanuel (2004). Lactose was determined by an enzymatic method, using the Megazyme (Megazyme International Ireland Ltd., Bray, Ireland) kit K-LACGAR. Ashes were determined by dry ashing the samples in a muffle furnace at 550 °C (AOAC, 2003b). The pH, determined by a pH meter (Beckmann Φ40, Beckmann, RIIC Ltd, High Wycombe, UK) was *ca.* 6.55 and titratable acidity, determined according to the AOAC Official Method no. 920.124 (AOAC, 2003c), was 0.125% (g of lactic acid/100 g of milk). Goats were grazed on pasture and milking was carried out twice daily, manually. After milking, the milk was immediately refrigerated to *ca.* 4 °C and within 3 h stored in a freezer (Ignis ICF410, Whirlpool, Siena, Italy) at −20 °C until used.

Lactic acid bacteria (*S. thermophilus* CR12, *Lactobacillus casei* LC01, *Lactobacillus helveticus* PR4, *L. acidophilus* 2949 and *Lactobacillus plantarum* 1288) belonging to the Culture Collection of the Department of Plant Protection and Applied Microbiology, University of Bari, were routinely propagated in M17 (Oxoid Ltd, Basingstoke, Hampshire, UK) (streptococci) or MRS (Oxoid Ltd, Basingstoke, Hampshire, UK) broth (lactobacilli) at 30 °C (*L. casei* LC01 and *L. plantarum* 1288) or 37 °C (*S. thermophilus* CR12, *L. helveticus* PR4 and *L. acidophilus* 2949).

Sodium caseinate was produced from goats' milk as described by Minervini et al. (2003). The pectin preparation was provided by Lippolis srl (Putignano, Bari, Italy) and contained *ca.* 115 g kg^{-1} moisture and 770 g kg^{-1} pectin; the degree of esterification for pectin was *ca.* 63% (HMP). Threonine was purchased from Sigma Chemical Co. (Milan, Italy).

2.2. Manufacture of fermented milks

Taking into account that it is difficult to manufacture a fermented goats' milk endowed with a good consistency, at the beginning of the experimental work some trials were carried out with the aim to set a protocol for manufacturing a product with a good consistency coagulum. All the fermented milks (FMs) were manufactured according to the following steps: addition of sodium caseinate (15.0 g kg^{-1}) and pectin (2.5 g kg^{-1}) to improve coagulum consistency, and of threonine (0.8 g kg^{-1}) to increase the concentration of acetaldehyde; heat treatment (70 °C for 30 min, while agitating) to inactivate non-spore-forming pathogenic and spoilage microorganisms, and to improve the distribution of the ingredients; rapid cooling to 40 °C and inoculation with a single- or mixed-starter culture, as indicated in Table 1. In details, heat treatment of milk was conducted in a bain-marie, while milk temperature was monitored by inserting a penetration probe of a Dualtemp thermometer (TFA Dostmann Ltd, Reicholzheim, Germany). The time needed for the center of a 500 ml milk bulk to reach 70 °C was *ca.* 10 min. Subsequently, heat-treated milk was rapidly cooled (*ca.* 5 min) to 40 °C in a ice-bath. Starter lactic acid bacteria were prepared as follows: 24 h old cells cultured in M17 or MRS broth were used to inoculate (1%, v/v) reconstituted 10% (w/w) skim milk from Oxoid Ltd. (Basingstoke, Hampshire, UK). Cell growth in reconstituted skim milk was determined by measuring the optical density of the culture at 600 nm (Ultrospec 3000, Pharmacia Biotech, Uppsala, Sweden) after clarification by addition of sodium citrate (Exterkate, 1984). One single preculture for each starter was prepared and used, even for mixed started fermented milks. Twelve h-cultured skim milk was used to inoculate (1%, v/v) goats' milk to obtain approximately 7.0 log cfu g^{-1}. Inoculated milk was stirred, bottled and incubated at 30 or 37 °C, depending on the optimal temperature of the lactic acid bacteria inoculated (Table 1), until pH 4.6 was reached. Finally, FMs were cooled to 4–6 °C and stored at 4 °C.

Three batches of goats' milk were collected on different days and on each occasion were used for the manufacture of the eight types of FM.

Table 1
Lactic acid bacteria used as starters, incubation temperature and time needed to reach pH 4.6 for different fermented goats' milks.

	Lactic acid bacteria	Incubation temperature (°C)	Time (h)
FM1	*Streptococcus thermophilus* CR12 *Lactobacillus casei* LC01 *Lactobacillus helveticus* PR4	37	3.5
FM2	*S. thermophilus* CR12 *L. casei* LC01 *Lactobacillus plantarum* 1288	37	3.5
FM3	*S. thermophilus* CR12 *L. casei* LC01 *Lactobacillus acidophilus* 2949	37	3.5
FM4	*S. thermophilus* CR12	37	4.5
FM5	*L. casei* LC01	30	7.5
FM6	*L. helveticus* PR4	37	13
FM7	*L. plantarum* 1288	30	8
FM8	*L. acidophilus* 2949	37	5