Food Microbiology 57 (2016) 8–15

Contents lists available at ScienceDirect

Food Microbiology

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

Microbial dynamics during shelf-life of industrial Ricotta cheese and identification of a *Bacillus* strain as a cause of a pink discolouration

E. Sattin [a], N.A. Andreani [b], L. Carraro [b], L. Fasolato [b], S. Balzan [b], E. Novelli [b], A. Squartini [c], A. Telatin [a], B. Simionati [a], B. Cardazzo [b, *]

[a] BMR Genomics, Via Redipuglia 21a, Padova, Italy
[b] Department of Comparative Biomedicine and Food Science, University of Padova, Viale dell'Università, 16, Legnaro, 35020, Padova, Italy
[c] Department of Agronomy, Food, Natural Resources, Animals, Environment (DAFNAE), University of Padova, Viale dell'Università, 16, Legnaro, 35020, Padova, Italy

ARTICLE INFO

Article history:
Received 28 July 2015
Received in revised form
3 November 2015
Accepted 29 December 2015
Available online 31 December 2015

Keywords:
Ricotta
Shelf-life
Microbial community
Spore forming bacteria
Pink discolouration

ABSTRACT

Dairy products are perishable and have to be preserved from spoilage during the food chain to achieve the desired shelf-life. Ricotta is a typical Italian soft dairy food produced by heat coagulation of whey proteins and is considered to be a light and healthy product. The shelf-life of Ricotta could be extended, as required by the international food trade market; however, heat resistant microflora causes spoilage and poses issues regarding the safety of the product. Next-generation sequencing (NGS) applied to the Ricotta samples defined the composition of the microbial community in-depth during the shelf-life. The analysis demonstrated the predominance of spore-forming bacteria throughout the shelf-life, mostly belonging to *Bacillus, Paenibacillus* and *Clostridium* genera. A strain involved in spoilage and causing a pink discolouration of Ricotta was isolated and characterised as *Bacillus mycoides/weihenstephanensis*. This is the first report of a food discolouration caused by a toxigenic strain belonging to the *Bacillus cereus* group that resulted the predominant strain in the community of the defective ricotta. These results suggest that the processing of raw materials to eliminate spores and residual microflora could be essential for improving the quality and the safety of the product and to extend the shelf-life of industrial Ricotta.

1. Introduction

Ricotta is a soft, white, fresh dairy product with a slightly sweet flavour typical of Italy and Ibero-American countries, manufactured from bovine, sheep, buffalo or goat whey milk by heat-coagulation of the whey proteins. The production of Ricotta is a system of adding value to a cheesemaking residual, largely applied in Italy both at the artisanal or industrial level. Fifteen percent of the whey produced in Italy is intended for the production of Ricotta. The amount of Ricotta consumed can be estimated at about 55,000 tons (retail), roughly 7% of cheeses purchased. Moreover, sales data show that Italian consumers seem to prefer Ricotta prepackaged instead of as a bulk product sold over a cheese counter with attendant (Troiani, 2015). Due to its low fat and salt content, high protein content and easy digestibility, Ricotta could respond to the demands of consumers and the market for light and healthy products. Ricotta can be eaten as a soft cheese even if it is more frequently used as an ingredient in dishes and desserts.

The name is derived from the Latin word *re-coctus*, literally re-cooked or cooked twice. Ricotta production technology uses the principle of coagulation and precipitation of the whey protein (mainly globulin and albumin) favoured by whey acidification (pH < 4.6) and heating. Ricotta is manufactured by thermally treating the whey at 80–90 °C followed by the addition of lactic or citric acid (1.5–2.5%). Afterwards, the surfacing curd is collected in moulds to partially remove whey and cooling. Whey could be enriched with whole raw milk or cream (1–5%) and salt (0.5–1.5%) to increase the yield and improve the organoleptic characteristics (Salvadori Del Prato 1992, Mancuso et al., 2014).

Dairy products are characterised by a reduced shelf-life because they are an excellent growth medium for a wide range of micro-organisms (Quigley et al., 2013). The spoilage process is produced as a consequence of food contamination by bacteria and fungi in raw

* Corresponding author. Viale dell'Università, 16, Legnaro, 35020, Padova, Italy.
E-mail address: barbara.cardazzo@unipd.it (B. Cardazzo).

http://dx.doi.org/10.1016/j.fm.2015.12.009
0740-0020/

materials or during production steps. Many of these microorganisms can produce undesirable reactions that deteriorate flavour, odour, colour, and the sensory and textural properties of foods. In addition, some microorganisms can potentially cause food-borne illness (Lu et al., 2013; Remenant et al., 2015).

Ricotta has a high moisture content, high concentration of residual sugars, an initial pH above 6.0, and starters are not added in production. As a consequence of these properties, Ricotta has a limited shelf-life even under refrigeration (Hough et al., 1999; Martins et al., 2010). The Ricotta industrial production process that includes heat treatment applied during manufacture and a final pasteurisation step, inactivates most of the resident microflora and limits the post-processing contaminations. For this reason, the shelf-life of industrial Ricotta is considered to be between 20 and 40 days (Mucchetti and Neviani, 2006). However, with the globalization of the food trade and the distribution from centralized processing, the request for an extended shelf-life of food products is becoming essential and a pressing issue. On the other hand, spores and thermophilic bacteria resist the production process and, with the extension of shelf-life, enhance spoilage events and pose issues regarding the safety of the product. Thus the extension of shelf-life remains a challenging goal (Lücking et al., 2013; Postollec et al., 2012). The chance of spoilage and pathogenic microorganism growth and survival depends on extrinsic factors associated with production and storage conditions, but also on intrinsic factors such as the composition of the microbial community (Ledenbach and Marshall, 2009).

Knowing the composition of the food microbiome is very important for defining the safety and the quality of a food product. As a matter of fact next-generation sequencing (NGS) platforms is an interesting approach for food microbiology, allowing deep microbial community definition directly on food samples (Ercolini, 2013; Mayo et al., 2014). The analysis is based on the millions of sequence reads obtained in a single run of 16S rRNA gene amplicons. 16S rRNA sequences are clustered into similarity groups, defined as Operational Taxonomic Unit (OTU), and classified by comparison against 16S rRNA sequence database. The strong competition between manufacturers has resulted in sustained technical improvements and cost reduction of almost all NGS platforms, allowing a wider usage of these technologies and providing a more complete description of the microbial community and its interaction and evolution (Loman et al., 2012; Shokralla et al., 2012).

In the present study, the NGS approach was applied to evaluate the composition and evolution of the microbial community during the shelf-life of an industrial Ricotta. The NGS approach provides a very comprehensive view of the microbial population composition and demonstrates that the improvement and application of such techniques on food microbiology could be an excellent method to evaluate food microbiological quality in-depth. The identification of the critical steps of the production process could be essential to control microbial load and to suggest solutions for safe food production. During the shelf-life study, a package of Ricotta revealing a pink discolouration was analysed leading to the identification of the bacterial strains involved in the spoilage.

2. Materials and methods

2.1. Samples

Two lots of bovine Ricotta were supplied from the Ricotta factory “Elda” (Vestenanova, VR, Italy) in January 2014 (winter samples named W) and July 2014 (summer samples named S). Ricotta is produced at 90 °C and sealed in plastic food packages of 100 g just after a pasteurisation step (1 min at 80 °C). The recipe includes pasteurised whey, cream at final concentration of 20%, lactic acid 0.1% and salt 0.1%. Twenty packages of Ricotta W and 26 of Ricotta S were collected in the factory the day after the production, transported to the laboratory in refrigerated containers and stored at 8 °C in a refrigerated incubator (MPM Instruments, Bernareggio, Italy) for 60 days. The temperature of 8 °C was chosen as mild thermal abuse to simulate the condition frequently occurring to the product during its commercial life.

After 4, 11, 14, 21, 25, 32, 39 and 60 days of storage, three packages of Ricotta were analysed (except for 39_S for which four samples were analysed). Each Ricotta sample was named with the day from production, the lot (W and S) and a progressive number.

Sample 14_S3, presenting a pink discolouration, was processed with other samples, but an additional Ricotta package, 14_S4, was sampled and analysed at the same day.

2.2. Microbiological analysis

Twenty grams of Ricotta were added to 180 ml of buffered peptone water (BPW Biokar Diagnostics, Beauvais Cedex, France) and serially diluted in the same solution. Samples were analysed for total aerobic mesophilic microorganisms (Total Mesophilic Count, TMC) plating on Plate Count Agar with skimmed milk (milkPCA, Biokar Diagnostics). The plates were incubated at 30 °C for 24–48 h. For aerobic spore count (ASC), 10 ml of 1:10 diluted samples were treated for 10 min at 80 °C and plated in Plate Count Agar added with 0.2% Starch (sPCA, Biokar Diagnostics) and incubated at 30 °C for 2–5 days.

The Ricotta sample 14_S3 (presenting pink discolouration) was analysed also for yeast and mould counts on Oxytetracycline Glucose Yeast Extract Agar (OGYE; Oxoid Microbiology Products, Thermo Scientific, Waltham MA, USA) and incubated for 3–6 days at 25 °C; for *micrococcaceae* on Mannitol Salt Agar (MSA Oxoid Microbiology Products, Thermo Scientific); for lactobacilli on MRS agar (MRS agar Oxoid Microbiology Products, Thermo Scientific); and for *Pseudomonas* on Pseudomonas Agar Base (PAB; Oxoid Microbiology Products, Thermo Scientific) incubated at 22 °C for 24–72 h. Moreover, with the aim to recover pink colonies, the 14_S3 sample diluted in BPW was plated in Minimal Bacterial Medium Agar (MBM 0.7% K_2HPO_4, 0.3% KH_2PO_4, 0.05% tri-sodium citrate, 0.01% $MgSO_4$, 0.1% $(NH_4)_2SO_4$, 0.2% glucose and 1.5% agar) and a Tryptic Soy Agar (TSA Oxoid Microbiology Products, Thermo Scientific) medium. Two pink colonies were resuspended, each in 100 μl of buffered peptone water and inoculated with a sterile syringe in two packages of Ricotta. The packages were opened after 14 days of incubation at 8 °C.

2.3. pH and organic acids determination

The pH of Ricotta samples was determined using a Portamess pH-meter (Knick 910, Berlin, Germany) equipped with an INLAB 427 electrode (Mettler Toledo, Urdof, Switzerland).

With the purpose of identification and quantification of organic acids, the samples of Ricotta were finely ground and diluted in a ratio 1:5 with the mobile phase (Sulfuric acid 0.01 N), held at 40 °C for 20 min, homogenised for 5 min with the Stomacher, centrifuged at 10,000 rpm at 4 °C for 10 min, and filtered first through a Whatman 4 and then through a syringe filter of 0.45 microns. The organic acids were determined by liquid chromatography with a Bio-Rad HPLC system equipped with a titanium pump mod.1350T and UV detector with variable wavelength mod. 1706. The column was an Aminex HPX-87H 300 × 7.8 mm (Bio-Rad) kept at a temperature of 60 °C, and mobile phase 0.01 N sulfuric acid flowing at 0.6 ml/min. A sample volume of 20 ul was injected by an autosampler plus 717 WISP (Waters) and the data were acquired and