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Bacterial community dynamics during cold storage of minced meat packaged under modified atmosphere and supplemented with different preservatives

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ABSTRACT

Since minced meat is very susceptible for microbial growth, characterisation of the bacterial community dynamics during storage is important to optimise preservation strategies. The purpose of this study was to investigate the effect of different production batches and the use of different preservatives on the composition of the bacterial community in minced meat during 9 days of cold storage under modified atmosphere (66% O_2, 25% CO_2 and 9% N_2). To this end, both culture-dependent (viable aerobic and anaerobic counts) and culture-independent (454 pyrosequencing) analyses were performed. Initially, microbial counts of fresh minced meat showed microbial loads between 3.5 and 5.0 log cfu/g. The observed microbial diversity was relatively high, and the most abundant bacteria differed among the samples. During storage an increase of microbial counts coincided with a dramatic decrease in bacterial diversity. At the end of the storage period, most samples showed microbial counts above the spoilage level of 7 log cfu/g. A relatively similar bacterial community was obtained regardless of the manufacturing batch and the preservative used, with *Lactobacillus algidus* and *Leuconostoc* sp. as the most dominant microorganisms. This suggests that both bacteria played an important role in the spoilage of minced meat packaged under modified atmosphere.

1. Introduction

Minced meat is susceptible to microbial spoilage because of its high concentration of nutrients and high water activity (Ercolini et al., 2011). The deteriorative effects caused by bacterial growth are discolouration, off-odours, and slime production (Singh et al., 2011). The rate of deteriorative changes depends primarily on the meat composition, the hygienic practices during the grinding and packaging process, and the storage conditions (Limbo et al., 2010). Modified atmosphere packaging (MAP) is generally recognised as an effective method for food preservation. A typical modified atmosphere for storage of ground red meat is 80% O_2 and 20% CO_2 (McMillin, 2008). The positive effects of a high oxygen level (>50%) are related to colour retention of red meat, minimising drip losses and inhibition of anaerobic and microaerophilic microorganisms (Amanatidou, 2001). However, shelf life extension of meat also depends on the presence of CO_2 (Limbo et al., 2010; Belcher, 2006; Dhananjayan et al., 2006; Amanatidou, 2001), with concentrations of 20–40% commonly used to suppress microbial growth (Vihavainen and Björkroth, 2007). Bacterial spoilage of meat is generally caused by species such as *Brochothrix thermosphacta* and species from the genus *Pseudomonas*, as well as by members of the *Enterobacteriaceae* and lactic acid bacteria (LAB) (Pennacchia et al., 2011). Their abundance and contribution to spoilage is largely influenced by the storage conditions (De Filippis et al., 2013; Doulgeraki et al., 2012; Ercolini et al., 2011; Pennacchia et al., 2011; Borch et al., 1996). Bacterial spoilage of meat is commonly determined using culture-dependent techniques such as viable plate counts, assessing the bacterial load and viability in the sample

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(Degirmencioglu et al., 2012; Esmer et al., 2011; Limbo et al., 2010; Koutsoumanis et al., 2008). It is generally accepted that microbial spoilage of meat occurs when counts reach levels of 7 log cfu/g (Degirmencioglu et al., 2012; Koutsoumanis et al., 2008). This level is commonly found to be correlated with sensory deterioration, like off-odours and the presence of slime in vacuum or gas packaged meat products (Limbo et al., 2010; Rao and Sachindra, 2002). Viable counts, however, are not suitable to characterise the microbial diversity of food products and to investigate thoroughly shifts in the bacterial communities during storage (Doulgeraki et al., 2012; Ercolini et al., 2006). Indeed, culturable microorganisms represent only a small fraction of the entire microbial diversity, and hence the microbial diversity in terms of species richness and abundance is grossly underestimated by culture-dependent techniques (Wintzingerode et al., 1997). In contrast, a thorough analysis of the microbial community can be achieved by culture-independent methods (Justé et al., 2008), such as Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) (Doulgeraki et al., 2012). This technique has, for example, been commonly applied to investigate shifts in the bacterial community during storage of beef in different conditions (Ercolini et al., 2011; Pennacchia et al., 2011; Brightwell et al., 2009; Ercolini et al., 2006; Fontana et al., 2006). However, as techniques such as PCR-DGGE do not allow identification, sequence-based approaches such as 454 pyrosequencing (Margulies et al., 2005) are currently increasingly used for detailed characterisation of diverse microbial communities from different ecological niches, including, among many others, meat and meat products (De Filippis et al., 2013; Kiermeier et al., 2013; Xiao et al., 2013; Nieminen et al., 2012a, 2012b; Ercolini et al., 2011).

Whereas the dynamics of the bacterial community of beef products have been studied before, little to nothing is known about differences in microbial load and dynamics during storage among different production batches. In addition, the effect of different preservatives on the microbial diversity and numbers remains to be investigated. Hence, the aim of this study was to investigate the dynamics of the bacterial community of minced meat during cold storage under modified atmosphere as well as to investigate the effect of different preservatives. This was assessed on three batches of minced meat sampled at three different manufacturing periods, and on one batch of minced meat using four different preservatives. Culture-dependent and culture-independent techniques were used. Microbial counting was performed to determine the number of culturable bacteria present. In addition, 454 pyrosequencing was used to gain insight into the bacterial community dynamics during storage.

2. Material and methods

2.1. Experiments

This study included two separate experiments. The first experiment was performed to compare the dynamics of the bacterial community of minced meat during cold storage under modified atmosphere of different production batches produced over several months. Therefore, three batches of minced meat, originating from three different manufacturing periods (batch 1, 2 and 3 produced in May, June and August 2013, respectively) were used. In a second experiment, the effect of different preservatives on the dynamics of the bacterial community of minced meat during storage was investigated.

2.2. Meat samples

Minced meat samples were obtained from a local meat processing company (Geel, Belgium). The samples composed of 98% beef and inulin as a fat replacer. Additionally, all samples of the first experiment were supplemented with 2.0% of a default preservative applied by the meat processing company (NaL, Opti.Form® SA (56–59% (w/w) sodium lactate and 3.5–3.7% (w/w) sodium acetate), Brenntag N.V., Deerlijk, Belgium). In the second experiment, samples were supplemented with the default preservative (2.0% NaL) or one of three other additives. The other additives were 1.6% (w/w) potassium lactate (KL, Opti.Form® PPA plus (71.3–74.3% (w/w) potassium lactate and 4.9–5.5% (w/w) potassium acetate), Brenntag N.V.), 2.0% (w/w) spice extract (SE, Misocarine LR, Barentz, Zaventem, Belgium) and a combination of 2.0% (w/w) NaL and 0.08% (w/w) ascorbic acid (AA, Brenntag N.V.). According to the manufacturer, the spice extract was a natural product based on the fermentation of spices (onion) and glucose syrup by lactic acid bacteria and acetic acid bacteria.

2.3. Packaging and storage

Minced meat samples of 300 g were placed in a polypropylene tray (PS001117, 178 × 138 × 55 mm^3, ES-Plastic GmbH, Hutthurm, Germany) and sealed with a foil (Rockguard 12-4-PET, adhesive, and Rockpeel-45-HT-AF, Rockwell Solutions Ltd, Dundee, UK) using a tray sealer (E 365 VG, G. Mondini S.p.A, Cologne, Italy). All samples were packaged using a gas mixture of 66% O_2, 25% CO_2 and 9% N_2 by vacuum compensation (vacuum pressure of 200 mbar). The oxygen transmission rate of the foil was 8 $cm^3/(m^2.d.bar)$ at 23 °C. The ratio between the volume of gas and the volume of product was 2:1 (Sivertsvik et al., 2004). To investigate the effect of microbial differences between production batches, 23 packages were prepared for each batch. Five packages were allocated for monitoring the gas composition during storage (five biological replicates). The other packages were used for microbial analyses. At each sampling point, three packages of minced meat were analysed (three biological replicates). For the second experiment, 15 packages were prepared for each preservative. Again, five packages were allocated to evaluate the gas composition (five biological replicates). Two packages were used for the microbial analyses at each sampling point (two biological replicates). For the study to be representative for storage of the meat products by consumers, packaged samples were stored in a home type refrigerator (Inspire Freestone, Electrolux, set point for temperature 5 °C) for a period of 9 days. This storage period is more than the estimated shelf life of the products stored under these conditions (7 days). At the end of storage, discolouring of the meat occurred and a spoilage odour was perceived. The temperature was monitored during the entire storage period using a data logger (Escort iLog internal sensor, VWR International, Leuven, Belgium). The average storage temperature for the first experiment, concerning different batches, was 5.9 ± 0.8, 5.1 ± 1.1 and 5.8 ± 0.6 °C for batch 1, 2 and 3, respectively. For the experiment including different preservatives, the average temperature during storage was 5.5 ± 1.5 °C.

2.4. Gas analysis

During storage the headspace gas composition was measured with a gas analyser (Checkpoint O_2/CO_2, PBI Dansensor, Ringsted, Denmark). To measure the gas composition, the needle (diameter 0.5 mm) of the gas analyser was pierced through a septum (reusable type, diameter 15 mm, DPI Dansensor) placed on the foil of the tray. Gas analyses were performed at the start of the experiment (day 0), as well as after 1, 6, 7, 8 and 9 days of packaging.