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Quality changes in refrigerated stored minced pork wrapped with plastic cling film and the effect of glucose supplementation

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ABSTRACT

Meat spoilage greatly depends on meat composition and storage conditions. Microbial and biochemical changes in minced pork (100-g portions) wrapped with a polyvinyl chloride film during a 4-day refrigerated storage were studied. As glucose is the first substrate used by spoilage bacteria and when it is depleted bacteria could generate undesirable volatiles, the effect of the addition of glucose to minced meat was also studied. Three treatments were used: control (C), without added glucose, and low and high glucose concentration (L and H), 150 mg and 750 mg of glucose in 100 g of meat, respectively. Spoilage bacteria, pH, redox potential, colour, basic volatile nitrogen, glucose, organic acids, and volatiles were analyzed in both recently prepared and stored pork samples. Storage resulted in increased levels of lactic acid bacteria and glucose-derived short chain alkyl volatiles, and a decrease in redox potential and volatile aldehyde levels. The addition of glucose to meat did not affect the biochemical characteristics of stored minced pork.

1. Introduction

The spoilage of refrigerated meat is a complex phenomenon where microbial activity is considered to be the primary cause (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015; Nychas, Skandamis, Tassou, & Koutsoumanis, 2008). The growth of meat spoilage bacteria is influenced by meat composition, initial microbial population, manipulation, and storage conditions (Blixt & Borch, 2002; Casaburi et al., 2015). Common approaches for delaying meat spoilage and improving meat shelf life are good hygienic practices, low storage temperatures, and adequate packaging.

During the storage of meat under refrigeration, the low temperatures lead to the establishment of particular psicrotrophic bacteria associations (Tsigarida & Nychas, 2001), which soon reach deleterious levels resulting in unacceptable off-odours, slime production, and increased discoloration. The microbial population of refrigerated meat is mainly composed by *Pseudomonas* spp., cold tolerant *Enterobaceriaceae*, lactic acid bacteria (LAB; such as *Lactobacilli* spp., *Leuconostoc* spp., *Carnobacterium* spp., etc.), *Brochothrix thermosphacta*, and *Clostridium* spp. (Doulgeraki, Ercolini, Villani, & Nychas, 2012; Pennacchia, Ercolini, & Villani, 2011). The development of spoilage microbial associations and their metabolism greatly depend on meat composition, i.e., glucose, lactate, non-protein nitrogen, storage temperature and the type of packaging, specifically the composition of the gas at the surface of the meat, i.e., O_2 and CO_2 levels (Casaburi et al., 2015; Doulgeraki et al., 2012; Argyri, Mallouchos, Panagou, & Nychas, 2015). Glucose plays a double role: it is the first substrate used by meat spoilage bacteria and, when it is depleted, highly undesirable volatile compounds are formed from the catabolism of nitrogenous compounds by spoilage bacteria, i.e., *Pseudomonas* (Nychas et al., 2008).

Microbial growth in minced meat takes place both at the surface and deep within the mince itself, where tends to reduce the levels of O_2 and increase those of CO_2 during storage. These changes rely not only on the intensity of microbial respiration but also on the ability of those gases to diffuse and dissolve in meat, and the permeability to them through an eventual packaging material (Koutsoumanis, Stamatiou, Drosinos, & Nychas, 2008). The availability of O_2 and the levels of CO_2 in minced meat affect, in turn, microbial growth. The predominance of one microbial group over another in refrigerated meat largely depends on gas concentration and, thus, the packaging conditions.

Pseudomonas spp. seems to be the predominant microbial group in refrigerated minced meat stored under aerobic conditions (Lambropoulou, Drosinos, & Nychas, 1996: Skandamis & Nychas, 2001). When its population reaches a high level (10^7–10^8 CFU/g), the glucose and lactate concentrations are depleted and nitrogenous compounds begin to be metabolized resulting in off odours (sulphides, esters, amines) and slime, i.e., spoilage (Nychas et al., 2008). On the other hand, the presence of CO_2 and/or the limitation of O_2 in meat stored under vacuum conditions or under O_2-CO_2-enriched atmospheres can

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restrict the growth of *Pseudomonas* spp., promoting the growth of Gram-positive bacteria with less spoilage potential (Koutsoumanis et al., 2008). LAB and *Br. thermosphacta* seem to be the most abundant bacteria in minced meat stored under those conditions (Argyri et al., 2015; Nieminen et al., 2011; Skandamis & Nychas, 2001).

The main end product from glucose metabolism by LAB in both aerobic and anaerobic conditions is lactate; the amounts of lactate and other end products (i.e., acetate) depend on O_2 and glucose concentrations (Condon, 1983; Samelis, 2006). *Br. thermosphacta* also uses meat glucose as the main substrate (Labadie, 1999) and the main end products from glucose degradation are lactate and acetoin, with concentrations depending on the atmosphere composition, i.e., more acetoin is produced at high levels of O_2 (aerobic metabolism) and more lactate is produced under O_2-free or O_2-available and CO_2 enriched atmospheres (Pin, García de Fernando, & Ordóñez, 2002). The ability of LAB and *Br. theromosphacta* for using other substrates than glucose seems very limited, although some LAB can use amino acids (e.g., arginine) to support their growth when the glucose content is low (Casaburi et al., 2015; Labadie, 1999; Nychas et al., 2008).

Research on shelf life of minced meat stored under aerobic, anaerobic, or modified atmospheres, with or without glucose supplementation, were previously carried out (Argyri et al., 2015; Koutsoumanis et al., 2008; Lambropoulou et al., 1996; Nieminen et al., 2011; Skandamis & Nychas, 2001). However, no study has been found on the shelf life of minced meat portions overwrapped with cling plastic film. The use of these films for meat and meat preparations is common in butcheries, restaurants, and households because it is easy to use and inexpensive. The films are usually made from polyvinyl chloride (PVC) or low-density polyethylene, having high O_2 and CO_2 permeability. During the storage of minced meat wrapped with cling film, a consumption of O_2 and a formation of CO_2 due to microbial growth are expected, giving lower O_2 and higher CO_2 concentrations in meat in spite of the high film permeability. Therefore, the storage conditions of the minced meat wrapped with cling film, regarding gas concentration in it, would be different from those packaged under aerobic, vacuum or modified atmospheres. The aim of this study was to determine the microbial and biochemical changes, mainly those produced by spoilage bacteria, happening in minced meat overwrapped with a cling plastic film due to a 4-day storage period under refrigeration conditions, and the effect of glucose supplementation on these changes.

2. Materials and methods

2.1. Minced meat preparation and sampling

Three 1.2-kg batches of minced pork, labelled as batch I, II, or III, were produced on three different days in the Food Processing Hall of the Food Hygiene and Food Technology Department (University of León). Each batch was prepared from a pork loin purchased in the local market, after removal of subcutaneous fat, and by mincing the lean in a butcher's mincer using a 5-mm diameter sieve. Every batch was divided into three 400-g sub-batches, which were mixed manually for 2 min with (i) 8 ml of water (without added glucose; control sub-batch C), (ii) 8 ml of a 0.075 ml/g glucose solution (low glucose concentration: 125 mg/100 g; sub-batch L), or (iii) 8 ml of a 0.375 ml/g glucose solution (high glucose concentration: 750 mg/100 g; sub-batch H), respectively. The concentrations of added glucose correspond approximately to 0, 5 and 25 times, respectively, the amount of glycogen/glucose found in post-rigor mortis pork muscle tissue (24–30 mg/100 g; Choe et al., 2008).

After mixing, each sub-batch was divided into four 100-g portions. Two portions were sampled for analysis on day 0 (d0 samples). The other two portions were tightly overwrapped with two layers (double layer) of polyvinyl chloride cling film (O_2 and CO_2 permeability of 4000 and 35,000 ml/m^2, respectively, for 1 d at atmospheric pressure; Film Catering 300, Extrusa, Uharte Arakil, Spain) and stored at 4 °C for 4 days before subsequent analysis (d4 samples). One 100-g portion from d0 samples and another portion from d4 samples were used for microbiological [aerobic mesophilic bacteria (AMB), LAB, and *Pseudomonas*] and physico-chemical [pH, redox potential (Eh), instrumental colour] analyses. The other two portions from d0 and d4 samples were kept at −18 °C up to 2 months, thawed overnight at 4 °C and analyzed for chemical analyses (volatile basic nitrogen VBN, short-chain organic acids, and glucose) and the determination of volatile compounds. All the above-mentioned analyses were performed in the d0 samples from the control sub-batch C (C-d0 samples) and in C-, L-, and H-d4 samples; and glucose was also performed in L-, and H-d0 samples (in all samples).

2.2. Microbiological analyses

Samples of 25 ± 0.1 g of minced meat were homogenized with 225 ml of peptone water (0.1% peptone and 0.85% NaCl; w/v) for 2 min in sterile bags using a Stomacher-400 circulator (Seward, West Sussex, U.K.). Serial decimal dilutions were prepared, and 1-ml aliquots of the appropriate dilutions were spread in duplicate on Standard Plate Count agar (Oxoid Ltd., Basingstoke, U.K.) for AMB counts and on De Man-Rogosa-Sharpe agar (Oxoid) in double layer for LAB counts, and incubated respectively at 30 °C for 48 h and at 30 °C for 72 h. Furthermore, aliquots of 0.1 ml of the appropriate dilutions were spread in duplicate on *Pseudomonas* agar base (Oxoid) prepared with CN *Pseudomonas* supplement (Oxoid) for *Pseudomonas* spp. counts and incubated at 25 °C for 72 h.

2.3. Physico-chemical and chemical analyses

Instrumental colour was determined in triplicate on the surface of the minced meat portions after removing the plastic film using the CIE L*a*b* system with a CM-500 chromameter (illuminant D65, visual angle 10°, SCI mode, 11 mm of aperture for illumination, and 8 mm for measurement; Konica Minolta, Osaka, Japan). The L*, a* and b* colour coordinates and ratio of reflectance at the wavelengths 630 and 580 nm, as an indicator of myoglobin oxidation during storage (AMSA, 2012), were studied.

The values of Eh and pH were measured directly in a 50 g sample of minced meat in duplicate at room temperature (20 °C) using a Basic C20 pHmeter (Crison Instruments, Barcelona, Spain) equipped with a 32-61-redox platinum electrode and a 52-32-pH penetration electrode, respectively.

The volatile basic nitrogen VBN was determined by the Conway microdiffusion technique (Conway, 1950). In duplicate, 10 g of the minced meat samples were placed into a 100 ml flask with 40 ml of distilled water. The mixture was homogenized with an Ultraturrax T18 (IKA Labortechnik, Staufen, Germany) for 1 min at 14,000 rpm, and the homogenate was filtered through a Whatman no. 1 filter paper (Whatman International Ltd., Maidstone, England). Afterwards, VBN was determined following the procedure described by Min et al. (2007).

The contents of short-chain organic acids were analyzed in duplicate using an Alliance-Waters 2690 chromatograph, coupled with a Waters 717 Plus autosampler and a Waters 2487 dual Absorbance detector (Waters, Milford, MA, USA), and equipped with a 300-mm × 7.8-mm Bio-Aminex (HPX-87H) protected with a 3 cm × 4.6-mm Micro-Guard H+ cartridge (Bio-Rad, Hercules, CA, USA). The method was performed as described by Bruna et al. (2003) with minor modifications on the elution program (initial flow of 0.5 ml/min for 30 min, and then increased to 0.8 ml/min up to 40 min). Identification and quantification was achieved using sodium lactate, pyruvate, gluconate and acetate standards (Sigma-Aldrich Química, Madrid, Spain).

The sugar content was analyzed using the extraction and chromatography procedure previously described with the following minor changes: a flow of 0.6 ml/min for 25 min with the column at 60 °C and using a Waters 410 refractive index detector; glucose (Sigma-Aldrich Química) was used as the quantification standard.