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A novel colorimetric sensor array for monitoring fresh pork sausages spoilage

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ABSTRACT

We report herein the development of a new optoelectronic nose composed of seven sensing materials prepared by the incorporation of pH indicators and chromogenic reagents selective to metabolites into inorganic materials (UVM-7 and alumina). The nose has been applied to monitor fresh pork sausage ageing. Sausages were packaged together with the disposable array in a modified packaging atmosphere (30% CO_2, 70% N_2) and colour changes were measured at seven day intervals. Simultaneously, microbiological and sensory analyses were performed. Colour modulations of the chromogenic array were processed with standard analytical tools, including principal component analysis (PCA) and partial least squares (PLS). According to the PCA, the array was able to monitor the sausage spoilage process and to distinguish between each sampled day. Moreover, the PLS statistical analysis of the chromogenic data displayed a linear correlation between the predicted and measured values of the storage days, mesophilic bacteria (cfu g^{-1}) and the sensory score with regression coefficients of 0.9300, 0.9472 and 0.9381, respectively.

1. Introduction

Fresh pork sausages are mainly composed of muscle pork meat and a variable amount of fat, which are more or less coarsely minced, mixed with a variety of non-meat ingredients, such as salt, aromas, species, colouring agents and conservative additives depending on local preparations (Pearson & Gillet, 1996; Tarté, 2009). The sausage mixture is stuffed into natural casings and ready-made products have to be kept under cold storage conditions until consumed. Fresh sausages are more perishable than other types of sausages and have a shelf life of a few days when stored in air at chilled temperatures, especially in the absence of conservative additives. Due to high fat content, the comminuted nature of the raw materials, the high water contents and lack of thermal processing, such a product is prone to spoilage by both lipid oxidation and microbial contamination (Georgantelis, Ambrosiadis, Katikou, Blekas, & Georgakis, 2007). Moreover, processing operations, such as particle size reduction and salting, can alter the pro-oxidative/antioxidative balance of muscle foods by mixing oxidation catalysts with lipid, oxidising myoglobin, releasing protein-bound iron, and inactivating antioxidant enzymes (Decker & Xu, 1998; Mielche & Bertelsen, 1994; Mitsumoto et al., 1991). Moreover during the grinding operation, oxidation and microbial contamination are accelerated due to loss of intracellular exudates and an increased surface exposed to air (Torrieri et al., 2011). Spoilage is observed when food becomes undesirable for human consumption caused by organoleptic changes, including variations in appearance (slime, discolouration) or development of off-odours and/or off-flavours.

Nowadays, society is becoming increasingly aware of the importance of diet for health, hence any issue relating to food safety has a considerable impact on consumer behaviour and official policies. At the same time, consumers increasingly prefer high-quality easy-to-prepare products that are safe and minimally processed, with less additives and ingredients, and with a long shelf

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life. The meat industry is, therefore, looking for emerging technologies that can achieve these processing and storage goals. Several methods have been developed to prolong the freshness period, including use of thermal treatments, addition of conservatives (Georgantelis et al., 2007; Martínez, Cilla, Beltrán, & Roncalés, 2006; Roller et al., 2002; Scannell, Hill, Buckley, & Arendt, 1997) or exposure to radiation (O'Bryan, Crandall, Ricke, & Olson, 2008; Olson, 1998). However, the best accepted systems by consumers are probably based on food packaging performance. For the latter, one of the most common systems is to combine refrigeration and modified atmosphere packaging (MAP) (Jeremiah, 2001; McMillin, 2008). In MAP, packaging not only acts as a barrier against contaminants, but also plays a crucial role in the selection of spoilage microorganisms by increasing the shelf life of food. Unfortunately, MAP packages do not provide any individual knowledge of freshness by mere visual inspection to alert eventual alterations of the cooling chain or contamination during the packaging process. Hence, simple-to-use reliable methods for meat freshness assessment and/or microbiological quality would benefit both consumers and the meat industry.

Despite the accuracy of some conventional methods to determine meat degradation (microbiological techniques, sensorial panels, detection of metabolite concentrations such as ATP, glucose and derived compounds or biogenic amines) (Argyri, Doulgeraki, Blana, Panagou, & Nychas, 2011; Dainty, 1996; Ellis & Goodacre, 2001; Lovestead & Bruno, 2010; Mora, Hernandez-Cazares, Aristoy, Toldra, & Reig, 2011; Piggott, Simpson, & Williams, 1998; Vinci & Antonelli, 2002; Xu, Cheung, Winder, & Goodacre, 2010), they are usually destructive, time-consuming, employ expensive instrumentation, require qualified personnel or cannot be used for *in situ* determinations. These procedures are generally suitable for food safety agencies, but not for use in supermarkets, at consumers' homes or in each piece of meat. One such inconvenience or more can also be found in other methods whose application has been recently studied for the same purpose: ion mobility spectroscopy (IMS) (Karpas, Tilman, Gdalevsky, & Lorber, 2002), mass spectrometry (Mayr et al., 2003), IR, RAMAN and fluorescence spectroscopy (Ammor, Argyri, & Nychas, 2009; Argyri, Panagou, Tarantilis, Polysiou, & Nychas, 2010; Ellis, Broadhurst, & Goodacre, 2004, Ellis, Broadhurst, Kell, Rowland, & Goodacre, 2002; Grau et al. 2011; Karoui, Downey, & Blecker, 2010; Kohler, 2007; Panagou, Mohareb, Argyri, Bessant, & Nychas, 2011; Papadopoulou, Panagou, Tassou, & Nychas, 2011; Sahar, Boubellouta, & Dufour, 2011; Sowoidnich, Schmidt, Maiwald, Sumpf, & Kronfeldt, 2010), and electronic tongues and noses (Berna, 2010; Campos et al., 2010; Paixão, Cardoso, & Bertotti, 2007; Roeck, Barsan, & Weimar, 2008). Moreover, the use-by date incorporated into the package does not inform about the particular state of each package because it does not report improper meat treatment during distribution or differences in end users' handling. Another innovative approach includes time-temperature indicators (TTI) (Kerry, O'Grady, & Hogan, 2006; Smolander, Alakomi, Ritvanen, Vainionpaa, & Ahvenainen, 2004), which inform about any temperature above a limit through colour changes. Although some correlations can be established between temperature and freshness, they do not really provide information about the biochemical processes occurring in food. If we bear this particular aspect in mind, the development of disposable systems capable of being incorporated into the package to offer individual easy-to-interpret information on freshness for end users may be of much importance.

Among the techniques that develop easy-to-handle disposable systems, the use of chromogenic chemosensors is, perhaps, one of the most promising because they are usually cheap, versatile, can be printed on the package, colour changes can be easily measured using cameras or other image-capturing systems, and in certain circumstances, they may allow the naked eye detection of colour changes through transparent films. Few technologies are as advanced or as inexpensive as visual imaging and the apparition of smartphones and intelligent home appliances offer interesting in-field opportunities. Although some chromogenic indicators have been described, they are generally based on a single compound, and they have their limitations such as lack of specificity (offering false positives or false negatives) (Kerry et al., 2006; Kuswandi, Jayus Restyana, Abdullah, Heng, & Ahmad, 2012; Nopwinyuwong, Trevanich, & Suppakul, 2010). Additionally, the presence of certain target metabolites is not necessarily an indication of poor quality. More exact correlations appear to be necessary among target metabolites, product type and organoleptic quality and safety. Moreover, the possibility of false negatives is likely to dissuade producers from adopting these indicators unless a specific indication of authentic spoilage can be assured. Thus, although some attempts have been made in single analyte indicators (*vide ante*), the most promising, potent and versatile approach to be applied in complex matrices is the use of optoelectronic noses, built by an array of dyes able to offer information through suitable colour modulations (Anzenbacher, Lubal, Palacios, & Kozelkova, 2010; Janzen, Ponder, Bailey, Ingison, & Suslick, 2006; Lim, Kemling, Feng, & Suslick, 2009; Palacios, Nishiyabu, Marquez, & Azenbacher, 2007; Rakow & Suslick, 2000; Suslick, Feng, & Suslick, 2010; Wu et al., 2009). Indeed, in the last few years, use of arrays of non-specific sensors showing high cross-reactivity has proved a suitable approach to analyse complex systems, and a number of examples of electronic noses and tongues to monitor muscle foods ageing can be found in the literature (Barat et al., 2008; Boothe & Arnold, 2002; Gil et al., 2011; Gil, Barat, Escriche, et al., 2008; Gil, Barat, García-Breijo, et al., 2008,; Rajamaki et al., 2006), in contrast, examples of chromogenic arrays are scarcer (Huang, Xin, & Zhao, 2011). Based on the above issues, and following our general interest in developing colorimetric probes (Abalos et al., 2009; Climent et al., 2011; Climent et al., 2009; Ros-Lis, García, et al., 2004; Ros-Lis, Marcos, Martínez-Máñez, Rurack, & Soto, 2005; Ros-Lis, Martínez-Máñez, et al., 2004, Ros-Lis, Martínez-Máñez, & Soto, 2005; Royo et al., 2011), we report herein a prospective study of the use of an array of chromogenic indicators with different chemical recognition properties which have been applied to follow the evolution of fresh pork sausage ageing.

2. Experimental

2.1. Chemicals

Bromocresol purple, m-cresol purple, malachite green and aluminium oxide were purchased from Sigma Aldrich; analytical-grade solvents were acquired from Scharlab. All the reagents were used as received with no further purification. 2,6-diphenyl-4-(2-(4-N,N-dimetilaminophenyl)vinyl)-pyryliumtetrafluoroborate (García-Acosta et al., 2006), bis(4-dibutylaminophenyl)squaraine (Sprenger & Ziegenbein, 1968) and UVM-7 (El Haskouri et al., 2002) were synthesised according to known procedures.

2.2. General techniques and characterisation

The XRD and TEM microscopy techniques were employed to characterise mesoporous materials. X-ray measurements were taken in a Bruker AXS D8 Advance diffractometer using CuKα radiation. The transmission electron microscopy (TEM) images of the particles were obtained with a Philips CM10 operating at 20 KeV. The samples for TEM were prepared by spreading a drop of nanoparticles suspension in decane onto standard carbon-coated copper grids (200 mesh).