Meat Science 96 (2014) 5–13

Contents lists available at ScienceDirect

Meat Science

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

The effects of low salt concentrations on the mechanism of adhesion between two pieces of pork *semimembranosus* muscle following tumbling and cooking

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ARTICLE INFO

Article history:
Received 19 March 2013
Received in revised form 21 June 2013
Accepted 28 June 2013

Keywords:
Salt reduction
Cooked ham
Tumbling exudate
Breaking stress
Protein physicochemical properties

ABSTRACT

The aim of this research was to gain deeper insight into the effect of salt content on the adhesion between pieces of *semimembranosus* pork muscle bound by a tumbling exudate gel. Hydrophobic site number, free thiol and carbonyl content were measured in tumbling exudate and meat protein to evaluate the protein–protein interactions involved in the adhesion process. Proteins were far more oxidized in exudate than in meat, and under our experimental conditions, salt content increased protein bonding in the exudate but not in the meat. Breaking stress increased between non-salted meat and 0.8%-salted meat but did not depend on the protein physicochemical properties of the tumbling exudate. Modifying the meat surface by tumbling alone, tumbling and salting, or scarification had no effect on breaking stress. It is suggested that the break between the meat pieces occurred between the tumbling exudate and the meat surface due to weaker chemical bonds at this location.

1. Introduction

Today's excessive salt intake is a proven risk factor driving the increasing incidence of many diseases, chiefly cardiovascular disease (Strazzullo, D'Elia, Kandala, & Cappuccio, 2009). The health agencies all recommend cutting dietary salt levels. In Ireland, the UK and the USA, meat products account for 20% of total dietary salt intake (Desmond, 2006). However, salt also plays key technological roles (water retention, protein solubilization, flavor) during the production of cooked delicatessen meat, which means there is only a degree of leeway for reducing salt content before finished product quality gets altered. In cooked ham, muscle–muscle adhesion determines sliceability, which is a vital property for commercial sale. The adhesion process occurs during thermal treatment (heating + chilling) as the exudate proteins salted out during the earlier tumbling process start to gel together. Experimental technological tests show that lowering the added salt content is likely to affect the adhesion process. This study set out to investigate the influence of salt content on the mechanisms underpinning the adhesion process in superior-grade cooked ham, which under French regulations does not contain polyphosphate additives.

Generally speaking, the adhesion of two materials – organic or otherwise – results from the interplay of several processes. The three key processes involved are molecular bonding, mechanical coupling, and thermodynamic adhesion (Awaja, Gilbert, Kelly, Fox, & Pigram, 2009). Molecular bonding can intervene both within the adhesive and between the adhesive and the surface. Mechanical coupling is a process whereby the adhesive couples physically to microstructures at the substrate surface: a rough-surfaced material glues better than a smooth-surfaced material provided there are no air bubbles at the surface interface. Furthermore, the adhesion process strengthens as material–adhesive contact surface increases. The thermodynamic adhesion model states that interfacial tension should be minimized to ensure full adhesion. Adherent materials get separated by two types of failure: cohesive failure, which takes places inside the adhesive, and adhesive failure, which occurs at the adhesive–substrate interface (Cognard, 2003). In this study the adhesive is the tumbling exudate while the surface is that of a soft biological material pork meat.

The exudate that is extracted at the muscle surface during the tumbling process is 80% water, 10–14% proteins and 0.2–5% lipids (Kerry, Stack, & Buckley, 1999; Siegel, Theno, Schmidt, & Norton, 1978). Protein composition analysis shows that exudate contains both myofibrillar proteins (essentially myosin, actin and tropomyosin; Pioselli, Paredi, & Mozzarelli, 2011; Siegel, Theno, & Schmidt, 1978) and sarcoplasmic proteins (mostly creatine kinase, β-enolase and fructose-biphosphate aldolase A; Pioselli et al., 2011).

Myofibrillar proteins are the key drivers of exudate gelation. The thermal gelation of myofibrillar proteins is a three-stage process of dissociation, thermal denaturation, and aggregation (Roussel & Cheftel, 1990) that leads to the formation of a three-dimensional

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http://dx.doi.org/10.1016/j.meatsci.2013.06.029

network resulting from the balance between protein–protein interactions and protein–water interactions. Several studies have highlighted a link between heat-induced physicochemical protein modifications and the stiffness of the gel (Boyer, Joandel, Ouali, & Culioli, 1996; Liu, Zhao, Xie, & Xiong, 2011; Visessanguan, Ogawa, Nakai, & An, 2000). Liu et al. (2011) used Raman spectroscopy to show that three types of bonds play a key role in the structure, the stability and stiffness of shaping meat protein gel: hydrophobic bonds, disulfide bridges, and other covalent bonds. Although it is implicitly assumed that an increase in exudate gel rigidity leads to stronger adhesion between pieces of ham, a causal link has never been formally elucidated.

Salt content has little influence on the protein content of the exudate (Siegel, Theno, & Schmidt, 1978), and its effect on myofibrillar protein gelation remains under debate: some teams assert that myofibrillar protein gel stiffness increases with increasing salt content (Park, Brewer, McKeith, Bechtel, & Novakofski, 1996), whereas other teams find the reverse effect (Hermansson, Harbitz, & Langton, 1986). The salt effect is in fact dependent on several parameters, including type of proteins involved (myosin only or myosin plus actin and/or sarcoplasmic proteins) and pH (Lefevre, Fauconneau, Ouali, & Culioli, 2002).

Two types of studies have investigated the effect of salt on muscle protein-binding adhesion between meat pieces. “Laboratory” trials have been conducted to analyze the ability of purified proteins (myosin, actomyosin in the presence or absence of sarcoplasmic proteins) to bind unsalted and non-tumbled meat pieces. The effects on breaking stresses were measured by the tension testing (MacFarlane, Schmidt, & Turner, 1977; Siegel & Schmidt, 1979). “Pilot” trials have studied the impact of amount of salt added in the tumbling process on the sliceability of cooked ham measured by either tension testing (Theno, Siegel, & Schmidt, 1978) or compression testing (Boutten, Ripoche, & Vendeuvre, 1998).

In “laboratory” studies, Siegel and Schmidt (1979) and MacFarlane et al. (1977) measured the ability of purified myosin to bind two unsalted meat pieces while varying the myosin extract salt content between 0 and 6%. Siegel and Schmidt (1979) found no difference in breaking stresses at 0 to 2% added salt content in the protein solution but increasing breaking stresses at higher salt contents. On the other hand, MacFarlane et al. (1977) found that breaking stresses increased sharply between 0 and 0.2 M NaCl (which equates to 0 to 0.9% salt in meat containing 75% water) but without change after this level. It thus emerges that although there is a consensus that increasing the salt content in the myosin extract increases breaking stress, there is divergence over the salt levels at which breaking stress starts to increase. For actomyosin, breaking stress does not vary from 0 up to 0.6 M NaCl (which equates to 0 to 2.6% salt in meat containing 75% water) but then increases as salt content increases up to 1.4 M NaCl (≈6.1% salt; MacFarlane et al., 1977).

The “pilot” studies show that in ham, a 0 to 2% variation in salt content facilitates adhesion whereas further increases up to 3% have no effect (Theno et al., 1978). Theno et al. (1978) concluded that salt content had to be 2% or higher in order to yield acceptable sliceability.

A review of the literature reveals that while there is consensus on the effect of salt on muscle–muscle adhesion in ham, there is ongoing debate over the minimum salt content required to maintain adhesion and, crucially, over the mechanisms involved. The effect of salt content on myofibrillar protein gelation and the link between gelation and adhesion have still not been clearly established, despite intensive research into the gelation process. The same is true for slicing failure, as we still do not know whether it occurs inside the gel matrix (cohesive failure, due to less tight gelation?) or at the muscle–gel interface (adhesive failure, an effect of surface condition?). This study aimed to gain deeper insight into the effect of low salt contents (from 0 to 2%) on the mechanisms underpinning muscle–muscle adhesion in cooked ham. Physicochemical measurements were performed on tumbling exudates produced in-lab or industrially-produced exudates. These measurements provide clues to the chemical bonds that dictate the stiffness of the gels formed. The exudates were then used to run adhesion experiments with salted or unsalted meat pieces presenting potentially modified surfaces. Finally, breaking stress analysis was used to interpret the effect of salt on the mechanisms responsible for adhesion. Eventually, the aims of this paper can be summarized in three main questions: how did salt influence the in-gel and gel-to-muscle surface bonds? Was breaking stress dependent on salt content and modifications of the surface of the meat? What was the kind of breaking failure: adhesive or cohesive?

2. Material and methods

2.1. Meat piece tumbling and exudate recovery

Cubes measuring 30 × 30 × 30 mm (±1 mm) were cut from 12 pork *semimembranosus* muscle samples. The average weight of the cubes was 28.4 ± 2.6 g. Muscle water content was 74.1 ± 1.5% and muscle pH was 5.6 ± 0.2. The brines were composed of 0, 11.2, 16.5 or 22.0% NaCl (respectively 0, 1.92, 2.82 and 3.76 M NaCl), 0.11% sodium nitrite, and 3.0% yam (84.9% dextrose, 13.9% sodium erythorbate and 1.2% monosodium glutamate; La Bovida, France). The cubes were bagged by 6, and the bags were then added with 10% brine (by weight) and vacuum-sealed; for instance, 17 g of brine was added for 170 g of meat. The samples were tumbled for 15 h at 4 °C in cycles of 5 min tumbling at 7.5 rpm and 10 min at rest, giving a total of 2250 rotations; the diameter of the tumbler was 47 cm (STALE, France). At the end of this tumbling process, two cubes per bag were sampled to run the adhesion analyses, and the exudate was then recovered from the remaining meat surface and the bag walls by gently scraping them. Around 510 g of meat was tumbled for each treatment condition (*i.e.* for each salt content).

In parallel, industry cooked ham producers provided us with exudates sampled under regular industrial production conditions (without polyphosphates), including a number of exudates from low-sodium cooked ham production lines. Industrial production procedures vary from business to business, with differences in brine compositions, brine injection rates (8–10%) and number of rotations (1500–2700). The targeted salt contents in the hams varied in the range 1.4–1.9%.

2.2. Thermal treatment

Meat cooking procedure was similar to the one detailed by Ismail-Fitry, Paterson, Wilkinson, and Purchas (2011) with some differences in the sample size, the fiber orientation and the mold material. The tumbled or non-tumbled meat was retrimmed down to 30 × 25 × 12 mm cubes without touching the protein exudate on the 25 × 12 mm surface to be bonded. Special attention was carried on the fiber orientation that was running perpendicular to the 25 × 12 mm surface, as the fiber orientation has been reported to have a significant effect on the binding strength (Purslow, Donnelly, & Savage, 1987). During some of the experiments, unsalted and non-tumbled meat was coated with 15 to 50 mg of exudate on each of the 25 × 12 mm sides (*i.e.* 5–16.7 mg of exudate.cm^{-2}). In one of the trials, the meat surface was scarified (about 1 mm deep and 2.5 mm between two scarifying cuts) using a razor blade before being coated with exudate. A cooking set-up was developed specifically to facilitate the adhesion process and cook the samples under a reliably repeatable protocol. The samples were vacuum-bagged two-by-two, carefully ensuring the two 25 × 12 mm bonding surfaces were pressed as flat as possible. Vacuum-bagging made it possible to eliminate air contact between the two meat pieces and thus facilitate mechanical coupling. The vacuum-bagged samples were then inserted into an aluminum mold (26 × 26 × 100 mm) to ensure efficient heat transfer. The third dimension of the mold (100 mm)