

# **Quality of Processed Pork**

**Influence of *RN* genotype and processing conditions**

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## Abstract

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This thesis aimed at increasing the understanding of how genetic and environmental factors, such as *RN* genotype and tumbling, influence the sensory perception and technological traits of processed pork and how they relate to water-holding properties and tenderness.

In two trials, loins of different *RN* genotypes were cured-smoked, sensory assessed by a selected and trained sensory panel and tested for functionality. Additionally, loins were either tumbled or not tumbled during processing in the second trial. The *RN<sup>-</sup>* allele strongly influenced sensory perception in general. Despite the lower water-holding properties of meat from *RN<sup>-</sup>* carriers compared with non-carriers, processed meat from *RN<sup>-</sup>* carriers scored more tender, juicy and acidulous by the sensory panel. Regardless of this sensory profile the consumers preferred cured-smoked loins from non-carriers of the *RN<sup>-</sup>* allele. Tumbling resulted in favourable water-holding properties and enhanced tenderness, but only slightly affected juiciness. Nevertheless, consumers tended to rank non-tumbled loins as most liked. For an optimum product quality the process design has to be fitted to the present assembly of machinery, because even small changes in the process can greatly affect the product quality.

The ability to predict sensory properties technologically was tested. Intra-myofibrillar water, determined by LF-NMR  $T_2$  relaxometry related to the sensory attributes juiciness, acidulous taste and meat taste in cured-smoked loins. Generally, water located in the highly organised myofibrillar lattice was of greatest importance for the eating quality. During processing unappealing pores often appear, which were predicted with high precision by relative LF-NMR  $T_2$  population sizes and image analysis.

In a third trial, the content of glycogen in fresh and cooked loin and meat juice was determined. All glycogen was broken down in non-carriers of the *RN<sup>-</sup>* allele during the *post mortem* glycolysis, whereas a considerable amount remained in *RN<sup>-</sup>* carriers. Cooking further broke down glycogen, but intact glycogen was to be found in the cooked meat and the fluid lost during cooking.

*Keywords:* porcine, meat quality, *RN* genotype, tumbling, water-holding capacity, sensory perception, processing, cured-smoked loin

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***Personal Thoughts***

*When you are a Bear of Very Little Brain, and you Think of Things, you sometimes find that a Thing which seemed very Thingish inside you is quite different when it gets out in the open and has other people looking at it.*

from Winnie-the-Pooh's Little Book of Wisdom, A.A. Milne & E.H. Shepard

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# Appendix

## Paper I-V

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

- I. Hullberg, A. & Lundström, K. 2004. The effects of *RN* genotype and tumbling on processing yield in cured-smoked pork loins. *Meat Science*. *In press*.
- II. Hullberg, A., Johansson, L. & Lundström, K. 2004. Sensory perception of cured-smoked pork loin from carriers and non-carriers of the *RN* allele and its relationship with technological meat quality. *Journal of Muscle Foods*, *Accepted*.
- III. Hullberg, A., Johansson, L. & Lundström, K. 2004. Effect of tumbling and *RN* genotype on sensory perception of cured-smoked pork loin. *Submitted for publication*.
- IV. Hullberg, A. & Bertram, H. C. 2004. Relationships between sensory perception and water distribution determined by low-field NMR  $T_2$  relaxation in processed pork – impact of tumbling and *RN* allele. *Submitted for publication*.
- V. Hullberg, A. & Ballerini, L. 2003. Pore formation in cured-smoked pork determined with image analysis – effects of tumbling and *RN* gene. *Meat Science* 65, 1231-1236.
- VI. Enfält, A-C. & Hullberg, A. 2004. Glycogen, glucose and glucose-6-phosphate content in fresh and cooked meat and meat juice from carriers and non-carriers of the *RN* allele. *Manuscript*.

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## List of abbreviations

G-6-P	glucose-6-phosphate
LF-NMR	low-field nuclear magnetic resonance
non-carriers	$rn^+/rn^+$ (wild type of the <i>RN</i> alleles)
PC	principal component
PCA	Principal Component Analysis
pH <sub>u</sub>	ultimate pH
PLSR	Partial Least Squares Regression
<i>PRKAG3</i>	( <i>RN</i> ) locus on pig chromosome 15
pT <sub>2x</sub>	LF-NMR relative relaxation population
RN	Rendement Napole
<i>rn</i> * allele	second mutant of the <i>PRKAG3</i> ( <i>RN</i> ) locus
<i>RN</i> <sup>-</sup> carrier	$RN^-/rn^+$ (with Hampshire as terminal sire)
T1	trial 1
T2	trial 2
T3	trial 3
<i>T</i> <sub>2</sub>	LF-NMR transverse relaxation
T <sub>2x</sub>	LF-NMR relaxation time constant
WB	Warner-Bratzler shear force
WHC	Water-holding capacity

## Introduction

Meat consumption has increased markedly the last decades in the western world. In Sweden, about 96% of the population eat meat (Svensk Köttinformation, 2003) and the meat consumption has increased about 5 % during the last year. It is now ~63 kg/person and year, of which ~36 kg originate from pig (Eva Stenberg, LRF, personal communication). At the same time as the increase in meat consumption, consumer demands on the quality of meat have risen. It is shown that consumption of pork is related to consumer satisfaction of pork (Bryhni *et al.*, 2003). Consequently, a high pork quality stimulates consumption, which is encouraging for the meat industry. However, the term 'meat quality' includes a variety of different aspects, the most important of which are hygiene, toxicology, nutrition, technology (function) and sensory (eating) quality. Which aspect is most focused on depends on who is applying it, e.g. producers, industry or consumers, and in which context the concept meat quality is used. In the western countries, hygienic and toxicological meat quality generally has high standard, nonetheless outbreaks such as the BSE crisis greatly damage the confidence in meat and meat products. Moreover, consumers are only willing to buy meat and meat products with an acceptable eating quality; indeed, they desire meat products with visual appeal, high juiciness and high tenderness. This can sometimes be incompatible with the interests of the meat industry, which aims at optimal functional properties of the meat. One good example is the  $RN^-$  allele, present in pigs of the Hampshire breed or crossbreds with Hampshire. The  $RN^-$  allele outermost affects the water-holding capacity of the meat negatively, whereas the eating quality is superior regarding tenderness and juiciness.

Moreover, other factors influencing meat quality are of interest. Numerous features, both genetic and environmental, are involved in the final meat quality. As already mentioned, the  $RN^-$  allele with its major impact on the overall meat quality is one. Meat is frequently processed, making processing another important factor that considerably influences both technological and sensory quality. Mechanical treatment of meat during processing is known to favour its water holding, but the influence on the eating quality is not entirely clarified. Thus, this thesis aims at increasing the understanding of how genetic and environmental factors, such as  $RN$  genotype and tumbling, influence the sensory perception of processed meat and how they are related to water-holding properties and tenderness. The thesis focuses on whole meat and products made of whole meat only; mixed, minced, restructured and other kinds of meat are outside the scope of this thesis.

## Background

Meat is a complex heterogeneous food, but with a highly organised ultrastructure. The features affecting meat quality are numerous and they are often collinear, *i.e.* dependent on each other. It is therefore difficult to discuss one variable at any particular time. Nevertheless, this background starts with the technological or functional properties of meat relevant for this thesis and discusses the effects of processing and *RN* genotype on technological characteristics, before considering their effects on the sensory perception.

### Technological quality of meat

#### *Muscle composition*

Skeletal muscles are arrangements of bundles of multinucleated muscle fibre cells containing myofibrils. Each myofibril includes mainly two types of filaments, myosin and actin, which constitute the contractile part of the muscle. The main components of muscle tissue after *rigor mortis* are water (~75%), protein (~20%), lipids and to lesser extent carbohydrates, free amino acids, dipeptides, vitamins, minerals and nucleotides. Muscle proteins can either be of myofibrillar or sarcoplasmic or stroma type. Myofibrillar proteins represents ~60% of the muscle proteins and constitute the contractile lattice, whereas sarcoplasmic proteins represents about 30% and consist of myoglobin and enzymes involved in the energy metabolism. About 10% of the muscle proteins are stroma proteins mainly containing the structural connective tissue proteins collagen and elastin (Lawrie, 1991).

#### *Water-holding capacity*

The high water content in muscle and meat makes water an important parameter affecting meat quality. A high ability of meat to retain water is advantageous from several points of view. On the market meat is paid for according to its weight, and loss of fluid during storage and processing naturally leads to less end profit. Moreover, consumer purchase is based on previous knowledge and visual impression. Trays filled with purge are unappealing for the consumers and give the impression of low quality meat. Indeed, eating quality is affected by the water-holding capacity (WHC) of the meat, which is discussed further in the thesis. A good overall definition of the term WHC is 'the ability of meat to hold its own or added water during application of any force or treatments such as grinding, processing or cooking' (Hamm, 1960). This illustrates the complexity of the term. Although massive efforts have been made to clarify the mechanisms behind the WHC of meat, much research is still to be done.

To understand the mechanisms involved in the WHC of meat the form in which water occurs in the meat has to be clarified. Ablett and Lillford (1991) explained it clearly as “ *Many of the physical properties of the water in high water foods – meat, fish, protein and polysaccharide gels - are similar to those of bulk water, yet its ability to flow as a liquid is clearly hampered. This water may be considered to be ‘bound’ because it is held within the food structure, ... . However, this does not*

necessarily mean that the water is bound in the chemical sense, and the simplest explanation is that the water is physically encaged in the matrix – i.e. it is free to move over many molecular dimensions, but is restrained in the structure by capillary or impermeable barriers.” Thus, the WHC depends on this matrix, which in meat primary consists of the muscle proteins, whereof the myosin-actin lattice dominates. Water is believed to exist in three forms, even when no clear lines can be drawn between these forms (Figure 1). First, about 4-5% of the water is bound to macromolecules, because the water molecules have polar properties and therefore link with electrical charged reactive groups of the macromolecules. This water is tightly bound and is not easily affected by extrinsic factors. The second form of water is the so-called immobilized water. It is attracted by the bound water and becomes less the farther from the macromolecules it is. The amount of water immobilized is influenced by physical forces applied on the muscle. Third, the free water is held only by weak surface forces (Hedrick *et al.*, 1989).

The functionality of the meat is highly dependent on changes in the meat structure, and the two most important events in meat are the conversion of muscle to meat and processing. Essential changes take place during the *post mortem* development, such as pH decrease, protein denaturation and the attachment of cross-links during *rigor mortis*. During processing addition of additives, water and thermal heating are important for the WHC (Offer & Knight, 1988).

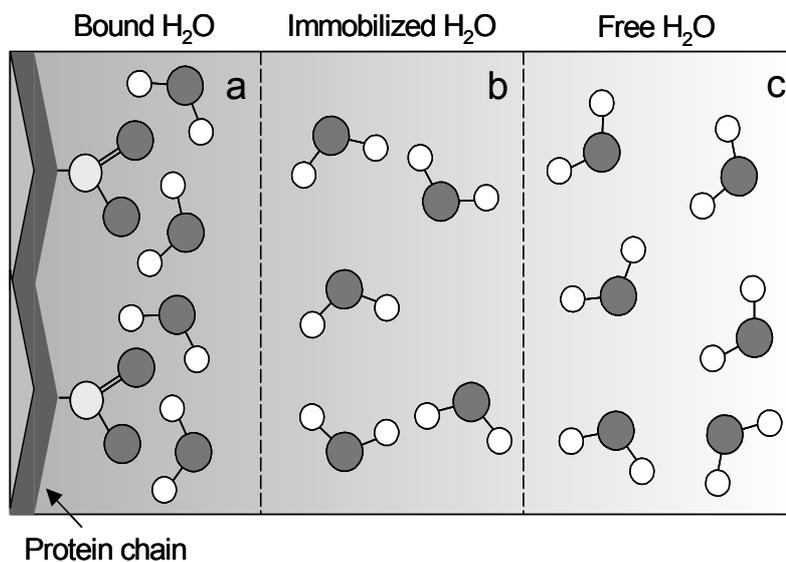


Figure 1. The different states of water in meat. a) The minor part of the polar water molecules bind to electrically charged reactive groups of the macromolecules in the meat and is referred to as the bound water. b) The immobilized form of water is structured, because this water is attracted by the bound water. c) Unstructured water held by capillary forces is free water (Modified after Hedrick *et al.*, 1989).

### *Conversion of muscle to meat*

After death the muscle is no longer able to utilize energy via the respiratory system. Energy is instead received through the glycolysis where mainly glycogen is converted to lactate. The accumulation of lactate causes the pH to fall and the glycolysis continues until a pH level is reached when the enzymes participating in the glycolysis are inactivated; thus, the relation between ultimate pH and glycogen content is not linear (Sahlin, 1978; Fernandez & Tornberg, 1991; Przybylski *et al.*, 1994). This plateau is normally at pH levels of around 5.5 (Lawrie, 1992). Hence, muscle glycogen levels at slaughter strongly influence final pH in the meat. However, the ultimate pH reached depends on several factors, *e.g.* type of muscle (Monin *et al.*, 1987a; Przybylski *et al.*, 1994; Fischer & Dobrowolski, 2001), genotype (Monin *et al.*, 1987a) and pre- and post-slaughter handling (Maribo *et al.*, 1988; Støier *et al.*, 2001). As pH is important for the water-holding capacity of the meat, attempts have been made to control the muscle glycogen content by feed (Gierus & Teixeira Rocha, 1997; Rosenvold *et al.*, 2001).

*Post mortem* glycolysis lowers the pH and thereby shifts the pH closer to its isoelectric point (~ pH 5.0). This is the point at which the electric charges on the amino and carboxyl groups on the proteins cancel each other. At this point the repulsive forces between actin and myosin filaments are nil and less water is held (Figure 2) (Hamm, 1960). Thus, at around pH 5.0 the WHC has its minimum. In addition, denaturation of proteins depends on temperature and acid condition. The closer the proteins to the isoelectric point, the more susceptible they are to denaturation (Warriss, 2000).

*Figure 2.* The effect of pH on water-holding properties of the intra-myofibrillar matrix in meat. Net charges are at minimum around pH 5.0.

### *Methods measuring the water-holding capacity*

Water can be lost in three different ways: by evaporation, as drip and during cooking (Offer & Knight, 1988). A variety of methods are available for measuring the WHC in meat and they are often used in combinations because they evaluate the water holding in different ways. Gravimetric methods such as drip loss from a slice of meat (Barton-Gade *et al.*, 1994; Honikel, 1998) and centrifugation (Bertram *et al.*, 2001a) are frequently used and give information on the meat's ability to retain water, which is of interest when studying changes during *post mortem* development, storage, ageing, cooking and processing. Weighing the meat before and after cooking provides further information of the WHC (Honikel, 1998). On the other hand, the total amount of water in the meat gives valuable information of the status of the meat and is easily calculated from the dry-matter content in the meat.

The above-mentioned methods are all limited in that they provide no information about where the water is located in the meat and how it is mobilized during changes *i.e. post mortem* development, ageing, storage, cooking and processing. Low-field nuclear magnetic resonance (LF-NMR) transverse ( $T_2$ ) relaxation measurement is a non-invasive method with potential to explore the state of water in meat (Lillford *et al.*, 1980; Bertram *et al.*, 2002). The mobility and compartmentalization of water and the compartments' reciprocal sizes can be determined by measuring the relaxation of water protons in the meat. Proton relaxation is normally in exponential form and from the decays obtained, relaxation time constants can be determined (Ruan & Chen, 1998). A multi-exponential decay is calculated out of several mono-exponential decays, each corresponding to one of the water compartments in the meat (Bro *et al.*, 2002). The number of exponentials the decay should be resolved into is still a subject of discussion (Bertram *et al.*, 2001a). A higher number of exponentials results in general in a better fit, but because the two first constants explain most of the water signal, bi-exponential fitting analysis has been commonly used in LF-NMR investigations on muscle, calculating two compartments of water (Renou *et al.*, 1985; Cole *et al.*, 1993; Brøndum *et al.*, 2000). Distributed exponential fitting analysis has the advantage of not restraining the decay to a discrete number of exponentials and with this calculation method three water populations have been distinguished in pork meat (Bertram *et al.*, 2001a). Still, the interpretation of the populations found is also a subject of discussion. NMR transverse relaxation times are influenced by the motion of the water molecules, and the more restricted the water in the meat - *i.e.* the more it interacts with its surrounding - the more the relaxation times are reduced. Thus, free water has long relaxation times (Ruan & Chen, 1998). The population with the fastest relaxation time constant ( $T_{2b}$ , centred about 1-10 ms, constituting about 1-4% of the total signal) is assigned to water tightly bound to macromolecules (Belton *et al.*, 1972). Hence, the disunity concerns the intermediate and slowest relaxation populations. Several theories have been presented, whereof one hypothesizes that the transverse relaxation data reflect physical compartmentalization in muscle tissue. Thus, muscle cell membranes would act as physical barriers preventing water molecules to diffuse, thereby dividing the water into intracellular and extracellular populations (Cole *et al.*, 1993). Tornberg and Larsson (1986) found a high correlation between the size of

the relative transverse relaxation water population corresponding with the slowest time constant at 100-150 ms and the amount of water around the fibre bundles determined by microscopy; they argued for a relationship between this water population and the extracellular water. The largest compartment (reflecting 81% of the water) was concluded to be interfilamental or intracellular. Also, Belton *et al.* (1972) suggested the two slowest compartments to reflect the intracellular and extracellular water. However, studies where cell membranes in the meat were chemically disrupted showed no changes in compartmentalization, indicating that it is not water populations divided by cell membranes that are reflected by the relaxation decay (Bertram *et al.*, 2001b). Bertram *et al.* (2001b) showed with a series of experiments that the transverse relaxation decay provides spatial information and suggested that the population with intermediate time constant, called  $T_{21}$ , reflects water located inside tertiary and quaternary protein structures and other highly organized parts of the myofibrillar protein structure in the meat, e.g. actin and myosin filament structures, whereas the slowest relaxing population, called  $T_{22}$ , reflects the extra-myofibrillar water. The intermediate water population is located at around 35-50 ms in fresh meat and constitutes 70-96% of the water signal, whereas the slowest population centred around 70-200 ms constitutes 2-10% of the water (Belton *et al.*, 1972; Fjellkner-Modig & Tornberg, 1986; Larsson & Tornberg, 1988; Brøndum *et al.*, 2000; Bertram *et al.*, 2001b).

### *Tenderness*

When the access of ATP after slaughter is depleted the muscle can no longer be held in a relaxed state. Actin and myosin forms an irreversible actomyosin complex and the flexibility of the muscle is lost. This stiffness is gradually decreased and the meat is tenderised or aged. Proteolytic enzymes are mainly responsible for the tenderisation, and the far most dominating are calpain and cathepsins. This loss of muscle structure integrity during the tenderisation involves numerous changes in the ultrastructure, which will not be discussed further here. (For a review see Tornberg (1996)).

### *Instrumental measurements of meat tenderness*

The purpose of tenderness measurements of meat is to reflect sensory tenderness by imitating the forces during biting and mastication. The forces to be applied are shear, compression or tension forces. Several methods are available, but for whole meat the Warner-Bratzler shear test is the by far most used. Samples, either cores or squared strips, are sheared at right angle to the muscle fibre direction using a thin metal blade with a triangular or squared hole. Generally the peak force is registered, but computer software of today gives the possibility to calculate the area below the deformation curve, which reflects total work done (Honikel, 1998). Another method available is the compression test or penetrometer test. A flat-ended plunger is driven once or twice vertically, e.g. 80% of the way through a standardised meat sample. The penetration is conducted perpendicular to the muscle fibre direction. Peak force during first deformation is registered as hardness and the ratio of work done between the two deformation curves as cohesiveness (Honikel, 1998). When a meat sample is compressed, it deforms in more than just the direction of the applied force, which complicates the interpretation. A two-

sided cell holding the meat sample is sometimes used to deform the sample laterally in one direction only (Lepetit & Culioli, 1994) (see **Paper II** for schematic figure of the cell).

## Processing

Cooking, curing and smoking are the most important processes for the meat industry. Meat is processed not only to make it more palatable, but also to prolong the shelf-life (Pearson & Gillett, 1996). About 65-80% of the meat produced is processed to some extent (Andersen, 2000) and there is an endless variety of more or less processed products on the market. When discussing whole meat, cured-cooked products are the most common. First, cure is added and then the meat is heat treated, often in combination with smoking (Figure 3). Mechanical treatment such as tumbling is often performed in connection with curing to strengthen the effects of curing.

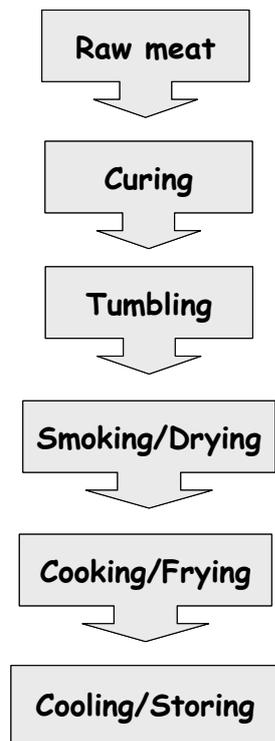


Figure 3. Processing steps of cured-smoked/cooked whole meat.

### *Curing*

Meat curing refers to the application of salt, nitrite, sugar, seasonings, phosphates and other additives to meat to develop products with unique properties. The advantages of salt were discovered a long time ago and the initial purpose of curing was to preserve meat and meat products. Nowadays, its major role is to enhance the eating quality, giving rise to a variety of meat products on the market, by the development of desirable tastes and flavours. Sodium chloride is the most frequently used additive, but nitrite and phosphates are other common curing agents (Pearson & Gillett, 1996).

Curing can either be done by rubbing the meat in pure salt and additives, or with the use of brine solutions, or in combinations. Salt crystals are not absorbed by the meat. NaCl has first to be solved in water or meat juice before salt diffusion into the meat can start (Pearson & Gillett, 1996). A commonly used method is to inject the brine into the meat with needles, even though saucing in brine is used, sometimes in combination with multi-needle injection. The advantage of multi-needle injection is the shortened processing time by enhanced brine diffusion. Because the needles are placed close to each other the brine has only to diffuse a short distance, instead of half-way through the meat chunk when brine immersed (Pearson & Gillett, 1996).

Meat proteins are solubilized by salt and phosphates (Offer & Knight, 1988), which is utilized in the processing of *e.g.* sectioned and formed meat. Salt and phosphate synergistically affect water-holding properties (Neer & Mandigo, 1977), which are further enhanced by mechanical treatment such as massaging and tumbling (Krause *et al.*, 1978b; Siegel *et al.*, 1978b; Theno *et al.*, 1978). Salt induces transverse swelling of meat fibres (Wilding *et al.*, 1986), which is due to depolymerisation of the myosin filament backbone (Offer & Knight, 1988).

Nitrite has been used as a curing agent for centuries and provides not only the stable pink colour of cured meat, but also a unique cure flavour (MacDougall *et al.*, 1975). Moreover, it improves oxidative stability and prolongs shelf-life (Skibsted, 1992).

### *Tumbling*

Equipment such as tumblers and massagers were first designed in Europe during the development of sectioned and formed meat. Generally, tumblers consist of rotating stainless steel drums of different types, causing chunks of whole uncooked pieces of meat, either fresh or cured, to tumble or drop, with or without the help of baffles (Pearson & Gillett, 1996) (Figure 4). This involves transfer of kinetic energy and friction, making the meat more pliable and drawing salt soluble proteins to the meat surface. Massagers are slow mixers, designed to stir large pieces of meat. They do not cause free falling of the meat and are therefore less rigorous than tumblers, but have similar advantages as tumblers (Pearson & Gillett, 1996).

The salt soluble proteins extracted during tumbling form a creamy, tacky exudate on the surface of the meat (Siegel *et al.*, 1978a). At heating the proteins in the exudate coagulates and form a protein-meat surface interaction. In processes where pieces of meat chunks are molded together or in cooked ham production where several muscles are to be held together, this coagulated exudate operates as a glue, increasing the muscle cohesion upon heating (Siegel *et al.*, 1978a; Siegel *et al.*, 1978b; Kartsaras & Budras, 1993) and favouring the slicing abilities and thereby lowering the waste. An extensive time of massaging results in a more pronounced adhesion compared with a short tumbling time and therefore, processing products as cured-cooked loins require a shorter tumbling time compared with sectioned and formed meat because the former products are constituted of mainly one piece of muscle.

In addition, cellular disruption of the meat tissue occurs during tumbling (Cassidy *et al.*, 1978; Lawlis *et al.*, 1992), which together with the curing additives allows the meat to improve the yield (Chow *et al.*, 1986). When the cell membranes are broken, migration of proteins to the surface can start, because salt and phosphates support protein solubility (Cassidy *et al.*, 1978). Constraining connective tissue sheaths around muscle fibres are disrupted, allowing further myofibrillar swelling introduced by salt (Wilding *et al.*, 1986; Kartsaras & Budras, 1993). The alteration of the cellular structure also promotes the incorporation of additives, for instance salt and nitrite and thereby favours a more rapid cured colour development in the meat (Krause *et al.*, 1978b).



Figure 4. Experimental tumblers used in trial 2 in this thesis.

Tumbling is often intermittent *i.e.* the meat is tumbled and rested in intervals, aiming at a balance between optimal tumbling time and time for the brine to diffuse. Even though several studies indicate the superiority of intermittent tumbling (Krause *et al.*, 1978a; Ockerman & Organisciak, 1978; Plimpton *et al.*, 1991) other suggests continuous tumbling (Gillett *et al.*, 1982). Cassidy *et al.* (1978) showed with histological studies that intermittent tumbling resulted in more alterations in cell structure than continuous tumbling, especially in the deep tissue. In addition, brine migration was superior in intermittent tumbled pork compared with continuous tumbling (Ockerman & Organisciak, 1978).

Nevertheless, other parameters during tumbling influence the final product quality too. Speed of the drum and total number of revolutions are important (Lin *et al.*, 1990), as well as the size of the drum (Pearson & Gillett, 1996) and the extent to which the drum is filled with meat (Pearson & Gillett, 1996). Temperatures of +0-5°C are preferred during tumbling, not only for hygienic reasons, but also because of a more favourable protein solubility (Iyimen, 1997). Because air and oxygen lower the protein exudates capacity to adhere meat pieces, vacuum is used during tumbling to reduce foaming and promote binding (Solomon *et al.*, 1980; Pearson & Gillett, 1996). Vacuum also increases cure absorption (Solomon *et al.*, 1980) as well as extraction of salt-soluble proteins (Sharma *et al.*, 2002).

### Cooking

Meat is cooked to increase the palatability and to ensure safe meat. Moreover, flavour is developed. Aroma volatiles developed in meat during cooking are mainly a result of Maillard reactions, which occur between reducing sugars, amino acids and degradation of lipids (Mottram, 1994).

Meat undergoes considerable structural changes upon heating (Figure 5). Generally these changes are due to heat-induced denaturation of the structural meat components such as collagen and myofibrillar proteins (Martens *et al.*, 1982). Two distinct increases in toughness have been observed when the temperature is increased during cooking. The temperature intervals when this toughening occurs differ somewhat between studies, but a first increase in toughening is observed at

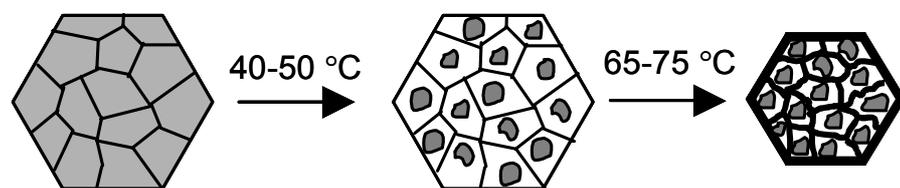


Figure 5. Effect of cooking upon shrinkage. Myofibrillar proteins denature at 45-50 °C and connective tissue between 60 and 75 °C. Black lines represent connective tissue and grey areas muscle fibres (Modified after Bailey, 1988).

around 40-50 °C (Davey & Gilbert, 1974; Bouton *et al.*, 1976) and a second increase at around 55-60 °C (Bouton *et al.*, 1976) or 65-75 °C (Davey & Gilbert, 1974). According to Davey and Gilbert (1974) the second phase is related to three events: toughening, shrinkage and weight loss. The first phase of toughening is suggested to correlate to the denaturation of the myosin, whereas collagen shrinkage is believed to cause the second phase (Davey & Gilbert, 1974; Bouton *et al.*, 1976). However, Martens *et al.* (1982) measured protein denaturation in beef cooked to various endpoint temperatures with differential scanning calorimetry. They reported that myosin caused toughening in the temperature range 40-60 °C, whereas actin strongly influenced toughening at 66-73 °C and collagen denaturated at 56 to 62 °C. Seuss and Honikel (1987) found a linear relation between sarcomere shortening in pork and cooking loss - above cooking losses of 10% - at final temperatures between 55 and 95 °C. They suggested the sarcomere shortening was due to heat-induced shrinkage of connective tissue.

### **RN genotype**

Normally, meat quality traits are controlled by an abundant number of genes whose individual effects are small compared to the total variance of this trait, so-called polygenes. More rarely, single genes with a large effect on the trait are discovered. Only two major genes with great influence on meat quality in pigs are identified so far, the Halothane sensitive gene and the Rendement Napole (RN) gene (Sellier & Monin, 1994). The halothane gene causes the syndrome malignant hyperthermia, activated by stress and exposure to the anaesthetic gas, halothane. This syndrome induces accelerated pH decline *post mortem*, influencing pork meat negatively (Sellier & Monin, 1994).

Pigs of the Hampshire breed or crossbreds with Hampshire were introduced by the farmer cooperation into the Swedish slaughter-pig population during the 70s to improve production, meat and carcass quality. The breed was excellent as terminal sire breed and Hampshire boars were widely used. However, it was discovered that meat from pigs of the Hampshire breed or crossbreds with Hampshire had inferior water-holding capacity compared with other breeds (Monin & Sellier, 1985). Meat from pigs of the Hampshire breed showed lower ultimate pH (Sayre *et al.*, 1963) and was called "acid meat". The lower ultimate pH was found in muscles with higher glycogen concentrations. The hypothesis of the existence of a major gene influencing meat quality was postulated by Naveau (1986) based on the distribution of the Napole yield, a standardized laboratory method to predict the technological yield of ham processing (Naveau *et al.*, 1985). The gene was called Remendent Napole (RN), after the Napole method, which in turn was named after the three researches Naveau, Pommeret and Lechaux, responsible for the Napole method (Naveau *et al.*, 1985). Later, Le Roy *et al.* (1990) confirmed the existence of the gene. Carriers of the  $RN^-$  gene or  $RN^-$  allele can either be homozygous or heterozygous for the allele ( $RN^-/RN^-$  or  $RN^-/rn^+$ ).

In comparison with other breeds, Hampshire pigs were discovered to have abnormally high glycogen levels in the muscles (Monin *et al.*, 1987a). The glycogen content in  $RN^-$  carriers was later found to be as much as 70% higher than in non-carriers (Estrade, M. *et al.*, 1993a; Estrade, M *et al.*, 1993b). Glycogen is an energy store accumulated in liver and muscle tissue, with higher concentrations in the liver than muscle, but more glycogen is stored in skeletal muscle because of its much greater mass (Stryer, 1988). It was shown that the excess of glycogen in  $RN^-$  carriers is located in the sarcoplasm of the white muscle fibres and that the sarcoplasmic compartment is abnormally enlarged in meat from  $RN^-$  carriers compared with non-carriers (Estrade, M. *et al.*, 1993a; Estrade, M *et al.*, 1993b). No difference in liver glycogen content was detected (Monin *et al.*, 1992) and therefore it can be concluded that the effect of the  $RN^-$  allele only includes muscle glycogen metabolism. Moreover, the morphology of the mitochondria revealed changes in mitochondria of  $RN^-$  carriers, indicating alterations in the glycogen metabolism of these pigs (Estrade, M *et al.*, 1993b).

Recently, two alleles present, the dominant  $RN^-$  and the recessive  $rn^+$ , were localised at the *PRKAG3* (*RN*) locus on pig chromosome 15 (Milan *et al.*, 2000). They showed that the mutation in the  $RN^-$  allele is a non-conservative substitutions in the *PRKAG3* gene, which encodes a muscle-specific isoform of the regulatory  $\gamma$  subunit of adenosine monophosphate-activated protein kinase (AMPK). The role of AMPK is to participate in the regulation of energy metabolism in eukaryotic cells. When activated the AMPK is expected to inhibit glycogen synthesis and stimulate glycogen degradation. Thus, the malfunction of the  $RN^-$  allele most probably causes defects in the glucose metabolism, either by inhibiting glycogen degradation or increasing glucose transport, and/or glycogen synthesis (Milan *et al.*, 2000).

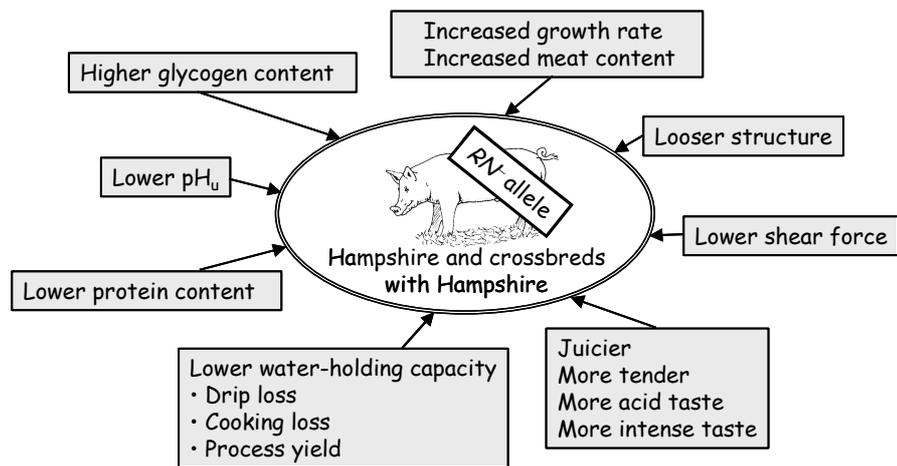


Figure 6. Effects of the  $RN^-$  allele on functional and sensory properties of meat.

The enlarged glycogen content in muscle of  $RN^-$  carriers severely affects overall meat quality. Due to the exceptional high glycogen content *post mortem*, the pH fall is prolonged, leading to ultimate pH levels around 5.4 (Lundström *et al.*, 1998b; Gariépy *et al.*, 1999; Nilzén *et al.*, 2001). Moreover, the water-holding properties are decreased because drip loss, cooking loss as well as technological yield are inferior (Enfält *et al.*, 1997b; Lundström *et al.*, 1998b; Gariépy *et al.*, 1999; Le Roy *et al.*, 2000). Meat from  $RN^-$  carriers contains less protein and subsequently more water (Monin *et al.*, 1992; Lundström *et al.*, 1998b). As the actin-myosin lattice is the main structure of water holding, the conditions for  $RN^-$  carriers to hold water are noticeably reduced compared with non-carriers. However glycogen has about the same ability to bind water as protein (2-4 times its own weight) (Greenleaf *et al.*, 1969; Olsson & Saltin, 1970), which to some extent may compensate for the lower protein content of  $RN^-$  carriers. Nevertheless, water is probably freed by glycogen during heating (Monin *et al.*, 1987b; Fernandez *et al.*, 1991) and thus meat from  $RN^-$  carriers loses more fluid during cooking (Lundström *et al.*, 1996) and processing (Enfält *et al.*, 1997a; Lundström *et al.*, 1998b; Gariépy *et al.*, 1999). Effects of the  $RN^-$  allele on meat quality are shown in Figure 6.

## Sensory quality of meat

Even within the field of sensory science the statement meat quality can be interpreted in several ways. Jul & Zeuthen (1981) defined meat quality as “the total degree of satisfaction that a meat gives the consumer”. Regarding liking of pork, juiciness, tenderness and absence of off-flavour were the most important sensory attributes among Scandinavian consumers (Bryhni *et al.*, 2003).

Meat tenderness depends of numerous factors of which some are listed here:  $pH_u$  (Guignot *et al.*, 1994), genotype (Jonsäll *et al.*, 2002), post-slaughter handling (Josell *et al.*, 2003b), endpoint temperature at cooking (Bouton *et al.*, 1976; Martens *et al.*, 1982; Wood *et al.*, 1995), cooking method (Schock *et al.*, 1970). Juiciness is as complex as tenderness and is highly dependent on, *e.g.* endpoint temperature at cooking (Heymann *et al.*, 1990; Wood *et al.*, 1995, Bejerholm & AASlyng, 2004), cooking temperature (Aaslyng *et al.*, 2003), genotype (Jonsäll *et al.*, 2002; Josell *et al.*, 2003a; Moelich *et al.*, 2003),  $pH_u$  (Guignot *et al.*, 1994; Eikelenboom *et al.*, 1996).

### *Effect of tumbling*

The sensory perception of tumbled meat is studied previously, but results are conflicting. Cassidy *et al.* (Cassidy *et al.*, 1978) reported differences in the ultrastructure after tumbling and suggested that an increased disruption contributed to a superior tenderness. Instrumental tenderness measurements confirm this hypothesis that less force was needed to deform tumbled meat [Chow, 1986 #149(Judge & Cioch, 1979), but sensory results diverge on this subject (Bedinghaus *et al.*, 1992; Lawlis *et al.*, 1992). The cellular alteration is also believed to influence juiciness (Dzudie *et al.*, 2000), but again sensory results vary between surveys (Siegel *et al.*, 1979; Chow *et al.*, 1986; Dzudie *et al.*, 2000).

### *Effect of RN genotype*

Although meat from  $RN^-$  carriers loses more water during heating fresh meat is most often scored as more tender, juicy and acid (Jonsäll *et al.*, 2000; Jonsäll *et al.*, 2002; Josell *et al.*, 2003c), even though not all surveys confirm a higher tenderness (Lundström *et al.*, 1996; Le Roy *et al.*, 2000) and juiciness (Lundström *et al.*, 1998b; Jonsäll *et al.*, 2001) in  $RN^-$  carriers. Moreover, fresh meat from  $RN^-$  carriers scored lower for crumbliness (Jonsäll *et al.*, 2002), higher for meat flavour intensity (Jonsäll *et al.*, 2001; Jonsäll *et al.*, 2002) and higher for odour intensity (Jonsäll *et al.*, 2001). Processed meat has been also been scored juicier and more tender (Johansson, M. *et al.*, 1998).

### *Sensory methods*

Numerous tests to evaluate the eating quality of foods are available, and the choice of test depends on what type of information is requested. International standards are provided that constitute general guidelines (ISO, 1985b) as well as comprehensive ISO guidelines for different tests.

The sensory tests available can be divided into two main groups, objective tests and subjective tests. The analyses of the objective tests all concern product properties, whereas the subjective tests (affective tests) concern the consumers' opinion (preference/liking) of the product. The objective tests then can be divided into two groups, descriptive tests and difference tests. The descriptive analysis is carried out by means of a selected and trained panel consisting of a minimum of 5 assessors. It is important to stress that the panel members should at all times be objective and that they disregard their likes and dislikes. The descriptive tests can be applied on several samples with many sensory characteristics on the same occasion. The results describe the sensory differences between samples and how large the difference is for each attribute. The difference tests merely answer questions such as: "Is there a difference between samples?", or "Is there a difference in tenderness?", a specific sample characteristic. Only one attribute at a time should be tested. When a general question is asked: "Is there a difference between samples?" there is no telling on what ground the assessments are given. The affective tests answer the question: "Which sample do you prefer/like the most?"

When using trained sensory panels the training is the calibration of the 'instrument'. Each panel member should get the opportunity to develop terms individually, which are then to be discussed in the panel and agreed upon [ISO, 1993 #599]. The training of the panel is of utmost importance. The descriptive sensory analysis comes last in a series of *e.g.* handling of a chunk of meat and it is the small sensory differences between samples that should be revealed. Therefore, the standardised sample preparations are crucial to the result. Difference analysis includes tests such as paired comparison test, ranking test, triangle test and duo-trio test, to ascertain whether samples differ (see ISO-standards for details, ISO 5495:1983, 4120:1983, 6658:1985, 8587:1988 and 10399:1991).

## Objectives

This thesis aimed at increasing the understanding of how genetic and environmental factors, such as *RN* genotype and tumbling, influence the sensory perception of processed meat and how they are related to water-holding properties and tenderness. The specific aims of the thesis were:

- *To determine the effect of RN genotype on sensory and technological quality of processed pork (Paper I & II).* Quality of fresh meat from the two *RN* genotypes is well documented, but because the predominating part of the pig meat produced is processed to some extent, it is of interest to determine the features of processed meat of different *RN* genotypes.
- *To investigate the effect of tumbling in combination with RN genotype on sensory and technological quality of processed pork (Paper I & III).* The process design considerably affects the product quality, but more knowledge is needed on the effects of tumbling on the eating quality. Tumbling alters the cell structure in the meat, which is believed to influence the two important sensory attributes, tenderness and juiciness. Besides, more understanding of how single processing steps affect meat quality of the two *RN* genotypes is desirable.
- *To study relations between water compartments established with LF-NMR  $T_2$  relaxometry and important sensory traits (Paper IV).* WHC is a fundamental quality feature in pork, but WHC and juiciness are not always positively correlated. It seems therefore that it is not the WHC of the meat, in terms of drip loss, cooking loss and processing yield that is most important for the eating quality, but the water distribution and availability in the meat.
- *To investigate the effects of RN genotype and tumbling regime on unappealing pore formation in cured-smoked pork determined with image analysis, in relation to conventional sensory analysis and visual scoring of images (Paper V).* Small pores are often observed in processed meat. *RN* genotype and tumbling affect the meat structure and could be expected to influence the formation of these pores. Further, because the assessment of the degree of pores solely is carried out by expensive sensory panels today, methods to reduce costs are welcomed.
- *To determine the fractions of glycogen and its degradation products in fresh meat, purge, cooked meat and cooking loss from carriers and non-carriers of the RN genotype (Paper VI).* High glycolytic potential in *RN*<sup>-</sup> carriers, in combination with the lower protein/water ratio is suggested to be the reason for the lower WHC of *RN*<sup>-</sup> carriers compared with non-carriers. Still, the mechanism is not clarified and further knowledge of glycogen in fresh and processed meat is needed, to understand the differences in WHC between *RN* genotypes.

# Material and Methods

## Material

The first five papers includes two studies referred to as trial 1 (T1) (**Paper I, II, V**) and trial 2 (T2) (**Paper I, III, IV, V**). T1 and T2 included loins from 62 (**Paper I**)/34 (**Paper II & V**) and 32 (**Paper I, III, IV, V**) randomly selected crossbred female pigs [Hampshire x (Swedish Landrace x Swedish Yorkshire)], respectively. The pigs were raised commercially and stunned by CO<sub>2</sub> and the average carcass weight was 81 kg (cold carcass without head and forefeet). The *M. longissimus dorsi* from the right half of the carcass in T1 and from both sides in T2 were collected 24 h *post mortem*. The part of the loin at the 5<sup>th</sup>-6<sup>th</sup> rib was used for quality measurements on fresh meat (pH, surface colour and drip loss) (Figure. 7) and the rest of the loin was processed to cured-smoked loin according to commercial routines in Sweden. The cured-smoked loins were divided into sections according to Figure 7, vacuum-packed, and stored at +4 °C until analysed, except for the section used for assessing the instrumental tenderness, which was stored frozen at -18 °C until analysed.

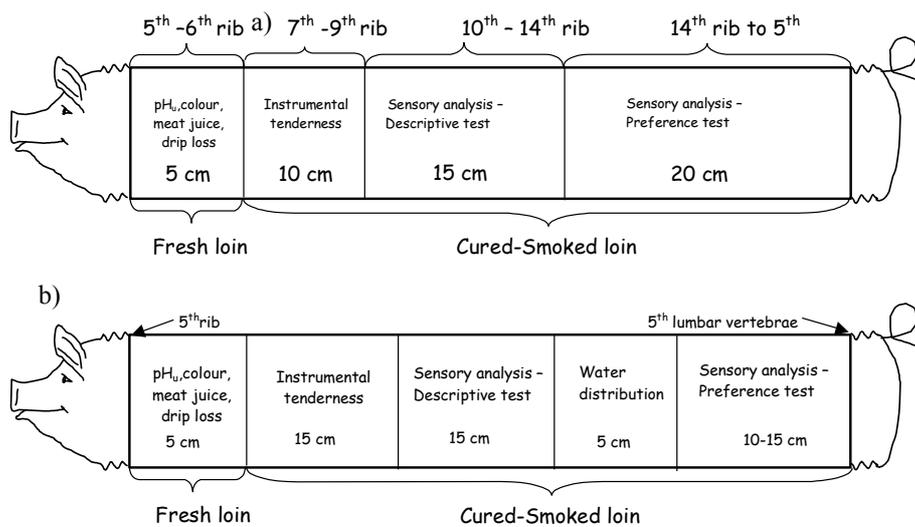


Figure 7. Schematic figure of the sampling of *M. longissimus dorsi*, indicating the approximate location along the vertebral column, a) trial 1, b) trial 2.

**Paper VI** included a third study, referred to as trial 3 (T3), including loins from 13 crossbred female pigs of the same crossbreed and with the same slaughter routine as above. *M. longissimus dorsi* from the right half of the carcass was collected 24 h *post mortem*. Ultimate pH ( $\text{pH}_u$ ) was measured at the last rib and loins with  $\text{pH}_u > 5.60$  were excluded, to avoid samples with DFD characteristics. The selected loins were transported from the cutting plant to the laboratory, on the same day, for further evaluation.

### Classification of *RN* genotype

For a preliminary classification, meat juice was collected for a rapid prediction of the *RN* phenotype. The concentration of glucose and glucose-6-phosphate (G-6-P) in meat juice were determined with a quantitative enzymatic method (Glucose (HK), Procedure No. 16-UV, Sigma Diagnostics) (T1 and T2) (Lundström & Enfält, 1997). On the basis of the bimodal distribution found, pigs with glucose and G-6-P concentrations  $\geq 40 \mu\text{mol/ml}$  meat juice were considered as carriers of the *RN* allele and loins with concentrations below this value were considered as non-carriers. In T3, approximately 5 g fresh meat was centrifuged (Simplex, Hereaus Christ GmbH, Osterode, Harz, Germany). A droplet (5  $\mu\text{l}$ ) of the released meat juice was used to determine the glucose concentration with a Glukometer Elite<sup>TM</sup> device (Bayer Diagnostics, Zürich). The animals were divided into *RN* carriers and non-carriers on the basis of the distribution found in earlier studies (Lundström *et al.*, 1998a; Nilzén *et al.*, 1999). Loins with glucose concentrations  $> 8 \mu\text{mol/l}$  meat juice were considered as *RN* carriers and loins with concentrations below this value were considered as non-carriers. To verify the phenotypic *RN* classification a genotyping was performed based on a DNA test using a PCR-amplification method to identify *RN* alleles (Milan *et al.*, 2000). In T1, 43 pigs were genotyped as carriers of the *RN* allele and 19 as non-carriers; in T2, 15 and 17 respectively. The distribution in T3 was 6 *RN* carriers and 7 non-carriers.

### Raw meat quality measurements

Technological meat quality in fresh meat was determined 48 h (T1 and T2) or 24 h (T3) *post mortem* as pH, drip loss (**Paper I & VI**) and colour (**Paper I**). Ultimate pH was measured using a portable Knick (Knick, Berlin, Germany) equipped with a combination gel electrode (SE104, Knick, Berlin, Germany). Water-holding capacity was measured as drip loss from 70- to 100-g slices stored horizontally 4 days on a grid (T1 and T2, **Paper I**) (Barton-Gade *et al.*, 1994) or hung for 2 days in inflated polythene bags (T3) (Honikel, 1998) in a standardised environment (+4 °C). Drip loss was calculated as the difference in slice weight before and after storing divided by the initial slice weight multiplied by 100. After at least 1 h of blooming, surface meat colour of samples was determined with a colorimeter (Minolta Chroma Meter CR-300, Osaka, Japan), using the  $L^*a^*b^*$  colour space, where positive values represented lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) (only in T1 and T2).

## Processing and processing yields

### *Trial 1*

Loins were processed 96 h post mortem at a commercial processing plant, as part of a commercial batch. They were cured by multi-needle injection with brine containing 16% nitrite saline (0.6% nitrite in the NaCl) to a quantity of about 16% of the initial loin weight. All loins were tumbled simultaneously for 4.5 h in a commercial tumbler, put in elastic nets, smoked and cooked during 4 h to an average internal temperature of 67 °C, and finally cooled to +4 °C. All transport and storing of the loins was performed in a cold environment. To determine the processing yields loins were weighed at each step of the process whereupon the yields were calculated according to the following formulas (1) (**Paper I**):

Yield (%), accumulative from step to step:

Yield after curing =  $b / a * 100$

Yield after tumbling and resting =  $c / a * 100$

Yield after cooking and smoking =  $d / a * 100$

Differences in yield ( $\Delta$ , %) between processing steps:

Yield difference between initial weight and curing =  $(b / a * 100) - 100$

Yield difference between tumbling/resting and curing =  $(c / b * 100) - 100$

Yield difference between cooking/smoking yield and tumbling =  $(d / c * 100) - 100$

Technological yield =  $(d / a * 100) - 100$

where  $a$  = initial weight at the slaughterhouse before transportation to the processing plant (g),  $b$  = weight after curing (g),  $c$  = weight after tumbling or resting (g) and  $d$  = weight after cooking and smoking (g).

### *Trial 2*

Loins were processed in an experimental processing plant. Most of the brine, containing 16% nitrite saline (0.6% nitrite in the NaCl), was added by multi-needle injection and the final brine content was adjusted by manual injection. To get an equal salt content in the final product, irrespective of tumbling treatment, loins to be tumbled were injected to 16% of initial loin weight and non-tumbled loins to 17% of initial loin weight. The loins from the right side of the carcasses were tumbled for 4 h at +8 °C. For this purpose three smaller tumblers were used with 5-6 loins per tumbler and occasion. The tumbling was carried out under constant vacuum (80-100 kPa) in intervals of 15-min work, with 8 revolutions per min, and 5-min rest periods. The two *RN* genotypes were, unlike in T1, tumbled in separate batches. Loins from the left carcass side were held in covered plastic trays at +4 °C while the other loins were tumbled (resting). All loins were put in elastic nets and then simultaneously smoked and cooked to an internal temperature of 68 °C for 3.5 h before cooled to +4 °C. All transport and storing of the loins was performed in a cold environment. To determine the processing yields loins were weighed at each step of the process and the yields were calculated according to the formulas (1) above (**Paper I**).

## Sensory analyses – descriptive test

In both T1 and T2, a descriptive test was carried out by a selected and trained sensory panel (ISO 8586-1, 1993) consisting of six members (**Paper II, III, IV & V**). The assessors were experienced at assessing pork. They were trained on assessing pork of different *RN* genotype, and trained specifically on cured-smoked loins from both *RN* genotypes for 6 h before profiling. The samples were served as two 4-mm-thick room-tempered slices placed in coded petri dishes. Only the centre of the slices was tested, i.e. the outer crust and fat layer were excluded. Six to eight samples were served in random order and replicated twice in two consecutive sessions. The assessments were performed in a room equipped with separate booths for each assessor and normal white light according to ISO (ISO, 1988a). Registration was performed with the PSA programme (PSA System/3. 2.09, 1994). To rinse between samples water and unsalted wafers were available. The attributes that the assessors unanimously agreed upon for T1 were tenderness, juiciness, acidulous taste, texture, meat taste, salinity, smoked taste, number of pores and homogeneity in cured colour, and for T2 initial tenderness, final tenderness, initial juiciness, final juiciness, acidulous taste, patty-like consistency, crumbliness, stringiness, meat taste, salinity, number of pores and homogeneity in cured colour. Attributes were assessed on a continuous scale from 0 to 100 with higher values indicating higher intensity of the actual parameter. Reference images were used in the assessing of number of pores.

## Sensory analysis - consumer test

In T1, a preference test with paired comparison (ISO 5495, 1983) was performed to evaluate the liking for cured-smoked loins from pigs of different *RN* genotypes (**Paper II**). The test included 136 consumers of different sex, age and status (students, military personnel and pensioners). Each consumer received two room-tempered slices of cured-smoked loin similar to the slices in the descriptive test, one from each *RN* genotype, and was asked about which sample he/she preferred. Samples were served in petri dishes coded with three-digit numbers and test persons were offered tap water for rinsing their mouths between samples. The cured-smoked loins used were from the same animals as in the descriptive test.

In T2, a consumer test based on ranking analysis (ISO 8587, 1988b) was performed to evaluate the overall liking of tumbled and non-tumbled cured-smoked loins from pigs of different *RN* genotypes (**Paper III**). The test was conducted in a large supermarket during a Saturday and included 144 consumers of different sex, age and status. Each consumer received four room-tempered slices of cured-smoked loin similar to the slices in the descriptive test, one from each of the combinations of tumbling treatment and *RN* genotype, and was asked to rank the samples according to overall liking (the higher the rank, the higher the liking). Samples were served as in the preference test.

In both consumer tests the consumer were asked - besides questions on sex, age and status - about their eating habits of cured-smoked loins. At the end of the preference test in T1 they were asked to write down why they preferred the chosen sample, whereas in the ranking test analysis in T2 they were asked to identify

among given attributes (tasty, juicy, tender, right degree of saltiness and mild smoked taste) what attributes they ranked the highest sample and they were also free to add their own attributes.

### **Sensory analysis – subjective scoring of images**

Images used for image analysis in T1 (see below) were scored subjectively for visible number of pores (**Paper V**). Six assessors judged each image visually at two occasions according to the number of pores on a scale from 1 (low intensity) to 5 (high intensity). Same reference images as for the sensory panel assessment of pores were used. Assessors had no previous experience on judging of images.

### **Salt and water content**

One slice of each cured-smoked loin (70-100 g) was trimmed from fat and crust and analysed for salt (T1 and T2) and water content (T2) (**Paper I, II, III & IV**). Only 30 loins (15 of each *RN* genotype) were analysed for salt content in T1. The salt content was measured with a chloride analyser based on conductivity titration (Corning 926, Chloride Analysator, Corning Ltd, Halstead, UK) in T1 and with a chloride titrator (CMT 10, Radiometer, Copenhagen) in T2. The water content was calculated based on analysis of dry-matter content.

### **Instrumental tenderness**

Warner-Bratzler shear force (WB) and compression force (CF) were measured at the 7th-9th rib using a Stable Microsoft System Texture Analyser TA-HDi (Godalming, UK) (**Paper II, III & IV**). Samples were thawed overnight at +4 °C and thereafter cut to strips with a 10 x 10 mm cross-sectional area with the fibre direction parallel to a long dimension of at least 50 mm. The WB and CF assessments were made 15 mm from each strip end, which made it possible to use both methods on every strip. At least 8 strips per loin were tested, holding a temperature of +15 to 20 °C during analysis. Mean values per animal (T1) and loin (T2) were used.

WB measurements were conducted with a shear blade with a rectangular hole, 11 mm wide and 15 mm high and a blade thickness of 1.2 mm, according to the method described by Honikel (1998). The maximum shear force (N/cm<sup>2</sup>) and total force (Nmm/cm<sup>2</sup>, area under the curve) needed to shear across the fibre direction was recorded at a test speed of 50 mm/min.

The CF measurements were conducted perpendicular to the fibre direction with a squared flat-ended plunger, 10 x 10 mm, driven at 50 mm/min twice vertically 80% of the way through the strip. The strips were placed in a metal cell fitted with two lateral walls along the long dimension of the strips (10 mm wide x 20 mm high x 50 mm long) so there was lateral strain in one direction only, *i.e.* down the fibre axis. The parameters recorded from the deformation curve were hardness (N/cm<sup>2</sup>, maximum force needed for first deformation) and cohesiveness (% the ratio between the total force needed during the second and first compression cycle times 100).

## Low-field NMR transverse relaxometry

Samples of cured-smoked loins were transported vacuum packed in a cold environment (+8 °C) from Sweden to Denmark for LF-NMR analysis (**Paper IV**). A sample (approx. 5 cm long and 1x1 cm in cross-sectional area, weight approx. 5 g) was taken along the fibre direction and placed in a cylindrical glass tube (14 mm in diameter and a height of 5 cm). This tube fitted into the NMR probe of 18 mm. Before measurement, the meat samples were thermostated to 25 °C in a water bath for 20 min. Three replicates per loin was taken from standardised regions of the loin and measured for water distribution.

The relaxation measurements were performed on a Maran Benchtop Pulsed NMR Analyser (Resonance Instruments, Witney, UK) with a magnetic field strength of 0.47 Tesla, with a corresponding resonance frequency for protons of 23.2 MHz. The NMR instrument was equipped with an 18-mm temperature variable probe.  $T_2$  was measured using the Carr-Purcell-Meiboom-Gill sequence (CPMG; Carr & Purcell, 1954). The  $T_2$  measurements were performed with a  $\tau$ -value (time between 90° pulse and 180° pulse) of 150  $\mu$ s. Data from 4096 echoes were acquired as 16-scan repetitions. The repetition time between two successive scans was 2 s. All relaxation measurements were performed at 25 °C.

Distributed exponential fitting analysis was performed on the  $T_2$  relaxation data using the RI Win-DXP programme (software release version 1.2.3) released from Resonance Instruments Ltd, UK. The RI Win-DXP programme performs distributed exponential curve fitting. A continuous distribution of exponentials for a CPMG experiment may be defined by equation (1):

$$g_i = \sum_{j=1}^m f_j e^{-t_i/T_j} \quad (1)$$

where  $g_i$  is the values of the exponential distribution at time  $t_i$ ,  $f_j$  is the pre-exponential multipliers of the distribution and  $T_j$  are the exponential time constants (the  $T_2$  values). The RI Win-DXP programme solves this equation by minimising the function:

$$(g_i - \sum_{x=1}^m f_x e^{-t_i/T_x})^2 + \lambda \sum_{x=1}^m f_x^2 \quad (2)$$

where  $\lambda \sum_{x=1}^m f_x^2$  is a linear combination of functions added to the equation to perform a zero-order regularisation as described by Press *et al.* (1992). This analysis resulted in a plot of relaxation amplitude for individual relaxation processes versus relaxation time. From such analyses, time constants for each process were calculated from the peak position, and the area under each peak (corresponding to the proportion of water molecules exhibiting that relaxation time) was determined by cumulative integration using an in-house programme written in Matlab (The Mathworks Inc., Natick, MA, USA).

## Image analysis for pore determination

Number of pores was determined with image analysis in T1 and T2 (**Paper V**). Cross-sections of cured-smoked loins were photographed (one side per slice of loin for T1 and both sides per slice of loin for T2) with a digital camera (Olympus C-1400L, Olympus Optical Co, Tokyo, Japan). The same exposure and focal distance were used for all images. Green was used as background colour. The meat pieces were lightened with two lamps. Polaroid filters were used on the lamps and camera to avoid specular reflections. Images were 1344 x 1024 pixel matrices with a resolution of 0.13 x 0.13 mm. The region of interest was interactively selected on the screen by removing meat edges, big breaks and other structures that were not of interest. The material provided was a set of colour pictures, but colour information was not used in the study. For simpler analysis, images were converted into grey scale by using the formula (Foley & van Dam, 1982):

$$f(x,y) = 0.35 f_R(x,y) + 0.58 f_G(x,y) + 0.07 f_B(x,y)$$

The objects to be analysed (pores) were dark on a lighter background. Because the background varied over the image it was corrected before the objects were analysed. The background was approximated by using median filtering, a non-linear smoothing method used for blurring and noise reduction. To perform median filtering in a neighbourhood of a pixel, the values of the pixel and its neighbours are sorted, the median is determined, and this value is assigned to the pixel (Gonzales & Woods, 1992). At this step the filter size was 50 x 50 pixels, spatially surrounding the central element ( $x, y$ ) that was included. An erosion operator removed the border effects created by the filter. The background variation was removed by subtracting the background image from the original image. An offset of 180 was used to avoid negative values. The difference image was computed as:

$$f_d(x,y) = f(x,y) - f_m(x,y)$$

The objects were segmented from the background by thresholding the images:

$$f_{\{\text{pores}\}}(x,y) = \begin{cases} 1 & \text{if } f(x,y) \leq T \\ 0 & \text{otherwise} \end{cases}$$

where  $T$  is a threshold value determined by histogram analysis techniques (Ballard & Brown, 1982). In the histogram two well-separated peaks can be observed, the right one representing background pixels and the left one representing pore pixels. The segmentation was performed by detecting the main valley between the two peaks and using the corresponding value as threshold. A size constrained labelling procedure was then applied to remove very small objects, which were probably noise and not pores. An area of 10 pixels ( $0.17 \text{ mm}^2$ ) was used as a minimum pore size. The number, size distribution, and spatial distributions of the extracted pores were measured.

### **Calculation of economic outcome**

The economic outcome was calculated in Euro (€) based on the present prices on the Swedish market for boneless cured-smoked loin (**Paper I**). The loss in income per kg fresh loin after processing, when the fresh meat comes from  $RN^-$  carriers instead of non-carriers or when tumbling is excluded, was calculated as the difference in final loin weight between treatments with an initial loin weight of 1 kg multiplied by the retail price per kg cured-smoked loin.

### **Cooking and cooking yield in T3**

In T3, samples (~150 g) for analyses of fresh meat were vacuum packed and stored in  $-18\text{ }^{\circ}\text{C}$  until analysis. Samples for cooking were weighed, vacuum packed, and stored at  $+4\text{ }^{\circ}\text{C}$  until 72 h *post mortem*. The samples were then reweighed and the meat juice released from the meat was collected and frozen. The meat was repacked in vacuum and cooked in water bath ( $+70\text{ }^{\circ}\text{C}$ ) for 2 h, until an internal temperature of  $+70\text{ }^{\circ}\text{C}$ , and then cooled for 30 min in cold running tap water. The weight after cooking was noted and the fluid released during cooking was collected and frozen. The cooked meat was vacuum-packed and frozen. The design of the trial is illustrated in Figure 11. Purge loss was determined as the percentage loss during storage in  $+4\text{ }^{\circ}\text{C}$ . Cooking loss was determined as the weight before cooking minus final weight divided by weight before cooking (**Paper VI**).

### **Glycogen, glucose and glucose-6-phosphate determination in T3**

Analysis of the total sum of glycogen, glucose and glucose-6-phosphate (G-6-P), as well as the sum of glucose and G-6-P was made in both fresh and cooked meat, and in fresh and cooked meat juice (**Paper 3**). The analysis was performed as described by Talmant *et al.* (1989) and Keppler & Decker (1970). Minced meat samples were homogenized with 0.6 M perchloric acid, on ice for 50 s. The homogenized sample was first hydrolyzed with amyloglycosidase, at pH 5 and  $37\text{ }^{\circ}\text{C}$  for 2 h, to split glycogen into glucose units. To inactivate the amyloglycosidase, 3 M perchloric acid was added. The sample was centrifuged, and the supernatant was used for the simultaneous determination of glucose, G-6-P and glycogen (ABX Diagnostics; Glucose HK 125- REF A11A00116). The sum of glucose and G-6-P was determined without the step of hydrolysing with amyloglycosidase. Glycogen content was calculated as the difference between the sum of glycogen, glucose and G-6-P and the sum of glucose and G-6-P. The result is expressed in  $\mu\text{mol}$  glucose equivalents per g muscle, wet weight for the muscle samples, and as  $\mu\text{mol}$  glucose equivalents per g juice for the meat juice samples. The content of dry matter was determined on minced samples and meat juice after drying at  $105\text{ }^{\circ}\text{C}$  for 16-18 h and 24 h, respectively.

## Statistical analyses

To eliminate the risk of confusion in the present thesis, Pearson correlation coefficients are referred to as correlations, whereas links analysed with multivariate methods are named relations.

Statistical evaluation was performed using the MIXED procedure, Statistical Analysis System, release 8.2 (SAS Institute Inc., Cary, NC, USA) (**Paper I, II, III, IV, V & VI**). The statistical models contained the fixed effects of *RN* genotype (T1 & T2) and tumbling treatment (T1) and when appropriate, the random effects of assessor and pig. Interactions between fixed effects were included in the model when significant. The initial weight of fresh loins were tested as covariate and accounted for when significant. In these cases, the Satterthwaite method was used to estimate the optimum degrees of freedom. Pearson correlation coefficients between treatments were calculated on mean values per animal or loin to avoid overestimation of the degrees of freedom. To get a clearer picture of the results of the sensory profiling the performance of assessors was evaluated statistically with eggshell plots and one-way ANOVA (PanelCheck, 1998, version 2.6, Matforsk, Aas, Norway). The software was described by Næs (1998). Overall data from the consumer test were analysed based on rank sums using the Friedman test described in ISO 8587 (1988b), whereas differences between highest ranked samples were tested with a conventional chi-square test.

Additionally, multivariate analyses were performed on mean values per animal (T1) or loin (T2) with the software The Unscrambler, version 7.8 (CAMO, Oslo, Norway) (**Paper II & IV**). Variable relationships and sample groupings were interpreted with principal component analysis (PCA) and presented as score and correlation loading plots. Partial least squares regression (PLSR) was used to explain the variation of each sensory (**Paper II & IV**)/technological (**Paper IV**) characteristic (*Y*-variable) by the variation of the genotype, instrumental and technological (**Paper I**) and variables in T1 and the variation of the  $T_2$  relaxation decays and distributed exponential fitted time constants/relative populations sizes (*X*-variables). Tumbling treatments and *RN* genotypes were included as *X*-variables in **Paper IV**, and when significant separate models were fitted including these *X*-variables. Cross validation was applied to optimise the PLSR models by determining the optimal number of PCs to be used. To evaluate the PLSR models the average prediction error was calculated as root mean square errors of prediction (RMSEP). Relevant *X*-variables were selected with the help of Marten's uncertainty test (Martens & Martens, 2000), regression coefficients and RMSEP values.

In T3, data were analysed using the GLM procedure, Statistical Analysis System, release 8.2 (SAS Institute Inc., Cary, NC, USA) (**Paper VI**). The statistical model used included the fixed effect of *RN* genotype.

## Summary of presented papers

### I. The effects of *RN* genotype and tumbling on processing yield in cured-smoked pork loins

The objective was to investigate the effects of *RN* genotype and tumbling treatment on yields throughout the processing of cured-smoked loins. Furthermore, the economic outcome was calculated for the different treatments because the technological yield is important for the meat industry. All loins in T1 were tumbled, whereas half of the loins in T2 were tumbled and the remainder was non-tumbled. Glucose and glucose-6-phosphate concentrations in meat juice and drip loss were higher, and ultimate pH and technological yield lower in loins of the *RN*<sup>-</sup> carriers than those of non-carriers (Figure 8). Water loss during processing was largest at heating, when yield between *RN* genotypes differed the most for T2. Contradictory, yield between genotypes differed the most at curing for T1. When tumbling was included in the processing the technological yield increased, but the meat with the *RN*<sup>-</sup> allele was still negatively affected. Salt content in cured-smoked loins was higher in non-carriers than *RN*<sup>-</sup> carriers in T1, whereas salt content in non-tumbled non-carriers was significantly lower than in the other cured-smoked loins in T2. Tumbled cured-smoked loins contained more water than non-tumbled loins, whereas no difference in water content was found between *RN* genotypes. There were moderate to high correlations between ultimate pH and processing yields except for curing yield. Curing yield was positively influenced by salt content, whereas water content in the cured-smoked loins was positively related to technological yield. The differences between the two trials suggest that the process design greatly influences the final product.

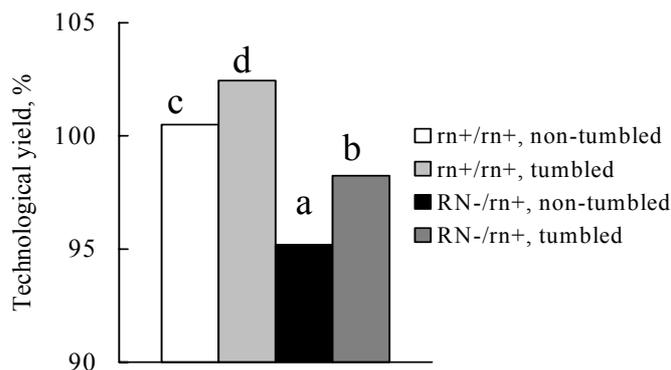


Figure 8. Technological yield in tumbled and non-tumbled cured-smoked loins of different *RN* genotypes in T2. Different letters above individual bars indicate significant differences between experimental groups ( $P < 0.05$ ).

## **II. Sensory perception of cured-smoked pork loin from carriers and non-carriers of the $RN^-$ allele and its relationship with technological meat quality**

The effects of  $RN$  genotype on the sensory perception of cured-smoked loins and corresponding technological parameters were investigated. Fresh meat was more reddish and yellowish, for  $RN^-$  carriers than non-carriers. Inferior water-holding properties in terms of drip loss and processing yield were detected in  $RN^-$  carriers, whereas instrumental tenderness measurements were superior related to non-carriers. Chemical salt content differed between  $RN$  genotypes, with  $RN^-$  carriers obtaining lower levels.

$RN^-$  carriers were scored more tender, juicy and acidic than non-carriers. They were also perceived as more patty-like and less salty. Further, they were more homogeneous in cured pink colour and tended to have more pores than non-carriers. Despite the superior eating quality in terms of higher tenderness and juiciness, 68% of the persons in the consumer test preferred cured-smoked loins from non-carriers. Multivariate statistical analyses showed that sensory-scored tenderness, juiciness, acidity, number of pores and homogeneity in cured pink colour were inversely related to instrumental tenderness measurements, but not well related to ultimate pH and water-holding capacity. Instrumental tenderness measurements explained 44% of the variation in sensory tenderness and  $RN$  genotype 41% of the variation in sensory juiciness.  $RN$  genotype best explained the variations in sensory attributes, followed by instrumental hardness. An intermediate to high correlation between tenderness/juiciness and most other sensory attributes was present.

### **III. Effect of tumbling and *RN* genotype on sensory perception of cured-smoked pork loin**

The study aimed at investigating the effects of tumbling and *RN* genotype on sensory perception of cured-smoked loins. Right-side loins were intermittently tumbled during 4 h, whereas left-side loins were left non-tumbled. Tumbled loins were more tender and uniform in cured colour as well as less acidulous in taste compared with non-tumbled loins. Further, the formation of undesirable pores was lower in tumbled loins. Final juiciness tended to be higher and meat taste intensity lower in tumbled loins, whereas initial juiciness did not differ. No significant interactions were present between tumbling and *RN* genotype for sensory attributes. *RN*<sup>-</sup> carriers were more acidulous in taste, had more pronounced meat taste and were saltier than non-carriers. Moreover, they tended to have higher initial juiciness and more undesirable pores, and be less homogeneous in cured pink colour. Juiciness and tenderness were highly correlated, as well as acidulous taste and meat taste. Except for the attributes 'number of pores' and 'homogeneity of cured colour', sensory attributes were generally intermediately to highly correlated with each other. Instrumental tenderness measurements corresponded strongly with sensory tenderness. *RN* genotype affected cured colour, whereas no effect of tumbling was observed on colour. Cured-smoked loins from *RN*<sup>-</sup> carriers were redder and more yellowish, as also observed in fresh meat from *RN*<sup>-</sup> carriers. Water content was positively influenced by tumbling treatment, but no differences in water content were found between *RN* genotypes.

No differences in consumer preference between the four treatments were detected in the ranking test, but most test persons ranked a non-tumbled loin highest regarding overall liking. The attributes most frequently used by test persons in the consumer test, to describe why their most liked sample was favoured were tasty, juicy and right saltiness. Less than half of the test persons mentioned tenderness as an important attribute. Instrumental tenderness measurements were well in line with sensory tenderness scores.

#### IV. Relationships between sensory perception and water distribution determined by low-field NMR $T_2$ relaxation in processed pork – impact of tumbling and $RN^-$ allele

The relationships between water distribution, measured with low-field NMR (LF-NMR) transverse ( $T_2$ ) relaxometry and sensory properties in tumbled and non-tumbled cured-smoked loins from pigs crossbred with Hampshire were investigated. Three populations centred at about 2, 40 and 600-800 ms were detected upon distributed analysis of the  $T_2$  relaxation (Figure 9). Clear differences in the characteristics of the intermediate population ( $T_{21}$ ) were observed between loins from carriers and non-carriers of the  $RN^-$  allele, which implies differences in water-protein interactions between the two genotypes. PLS regressions between NMR  $T_2$  variables and sensory attributes revealed significant relations between NMR  $T_2$  variables and the sensory attributes juiciness, acidulous taste and meat taste, which could mainly be ascribed to the  $T_{21}$  time constant. In addition, the number of unappealing pores assessed by the sensory panel was highly related to the relative  $T_2$  populations, implying that the microstructure is directly reflected in the NMR  $T_2$  populations. A low degree of explanation was obtained when attempting to predict the processing yield from NMR  $T_2$  variables. However, the relation improved when  $RN$  genotypes and tumbling conditions were included as predictors. Thus, obvious effects of tumbling treatments and  $RN$  genotypes were observed for the relationship.

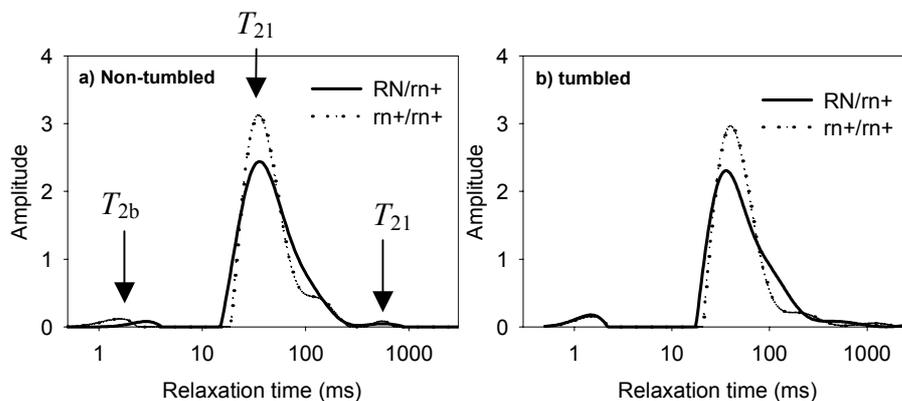
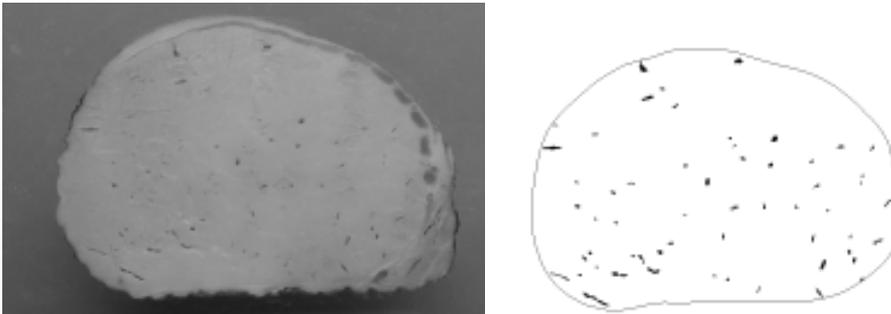


Figure 9. Representative distribution of LF-NMR transverse relaxations ( $T_2$ ) times for tumbled and non-tumbled cured-smoked pork loin with or without the  $RN^-$  allele.

## V. Pore formation in cured-smoked pork determined with image analysis – effects of tumbling and $RN^-$ gene

The objective of the present study was to investigate the effects of  $RN$  genotype and tumbling condition (tumbled or non-tumbled) on the number of undesirable pore formation in cured-smoked loins using image analysis (Figure 10). Even when not significant, loins from  $RN^-$  carriers contained more pores than loins from non-carriers. Tumbling clearly decreased the total number of pores, but increased the mean area per pore. Tumbled loins from non-carriers contained half the number of pores compared with tumbled loins from  $RN^-$  carriers. Even when  $RN$  genotype and tumbling influenced the number of pores, they did not explain the development of pores in processed meat. When pores were distributed according to size, it was found that the increase in number of pores mainly was due to formation of smaller pores. The high correlations found when comparing the image analysis results with data from a trained sensory panel ( $r=0.80$  and  $0.82$  in T1 and T2, respectively;  $P=0.001$ ) and visually scored images ( $r=0.90$ ;  $P=0.001$  in T1) indicate that image analysis is an excellent tool in this type of investigation. Ultimate pH was inversely related with pore formation, but only for non-carriers. Thus, the relationship seems only to be valid in a certain pH interval and no further increase in number of pores appears to take place at pH values below 5.4.



*Figure 10.* Pore formation in cured-smoked pork loin and pores obtained with image analysis.

## VI. Glycogen, glucose and glucose-6-phosphate content in fresh and cooked meat and meat juice from carriers and non-carriers of the $RN^-$ allele

The objective of this study was to investigate the content of glycogen, glucose and glucose-6-phosphate (G-6-P) in fresh and cooked meat and meat juice (purge and cooking loss) from carriers ( $RN^-/rn^+$ ) and non-carriers ( $rn^+/rn^+$ ) of the  $RN^-$  allele (Figure 11). The total content of glycogen, glucose and G-6-P was significantly higher in  $RN^-$  carriers compared with non-carriers both in fresh and cooked muscle, as well as in fresh and cooked meat juice. Also the sum of glucose and G-6-P was higher in all fractions for the  $RN^-$  carriers compared with non-carriers. Loins from the  $RN^-$  genotype had lower ultimate pH and higher drip, purge and cooking loss compared with loins from non-carriers. It can be concluded that in  $RN^-$  carriers, there is muscle glycogen left after the post mortem process, which is released with the meat juice during storage and cooking, lowering the level in the meat.

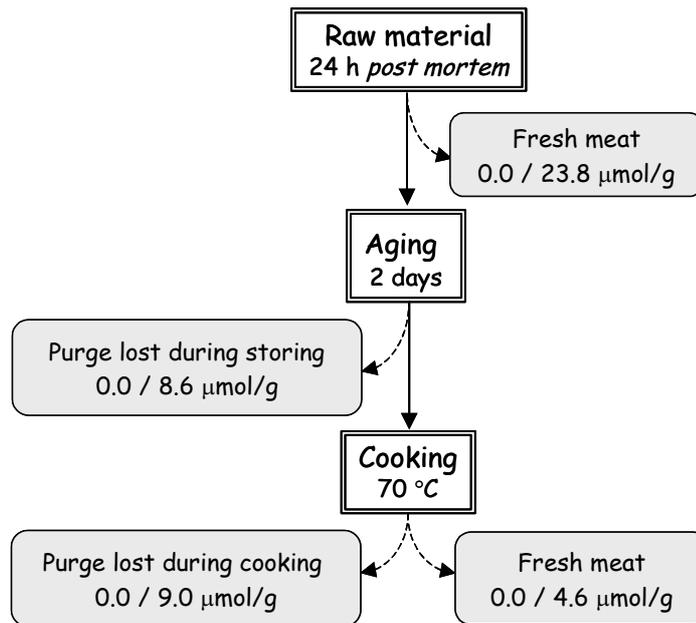


Figure 11. Illustration of the design of T3, with glycogen content in the different fractions given for each  $RN$  genotype ( $rn^+/rn^+$ ,  $RN^-/rn^+$ ;  $\mu\text{mol/g}$  meat or meat juice).

## General discussion

Meat contains about 75% water, which can be lost or gained to a higher or lower extent depending on the *pre*- and *post*-slaughter handling of the meat (Offer & Knight, 1988). Naturally, both functional quality and eating quality strongly depend on the WHC (Hamm, 1978). However, there are complex relationships among physiological and chemical variables regarding WHC, and the mechanism and the regulation of the WHC is not fully understood, particularly the relationship between WHC and sensory attributes. Today, it is though known, that the WHC of meat carrying the  $RN^-$  allele is inferior compared with non-carriers (Lundström *et al.*, 1998b; Gariépy *et al.*, 1999; Le Roy *et al.*, 2000)(**Paper I**) and other breeds (Monin *et al.*, 1987a; Enfält *et al.*, 1997b). Factors affecting this difference in WHC between  $RN^-$  genotypes are still discussed. The abnormal high glycogen levels in glycolytic or white muscles of  $RN^-$  carriers compared with non-carriers (Monin & Sellier, 1985; Fernandez *et al.*, 1992) contribute to the abnormally low ultimate pH in meat from  $RN^-$  carriers (Le Roy *et al.*, 2000)(**Paper I**). Low pH decreases the WHC because the electrical charges between myofilaments subside and transverse myofibrillar shrinkage takes place (Hamm, 1960). Because the lattice spacing mainly comprises structural proteins, such as actin and myosin (Offer & Knight, 1988), the protein content also influences the WHC. Muscle of  $RN^-$  carriers contain less protein than muscles of non-carriers (Monin *et al.*, 1992; Enfält *et al.*, 1997a; Lundström *et al.*, 1998b; Leuret *et al.*, 1999), which is suggested to further contribute to the inferior water-holding properties of this meat (Fernandez *et al.*, 1991). Additionally, myoproteins of meat from  $RN^-$  carriers are suggested to be more heat sensitive, thus they denature more easily upon heating (Deng *et al.*, 2002; Bertram *et al.*, 2004). This may further contribute to the higher cooking loss of  $RN^-$  carriers. Moreover,  $RN^-$  carriers are reported to have weaker structure, which could also contribute to the poorer WHC of this meat (Estrade, M. *et al.*, 1993a; Monin, 1995). In summary, it can be concluded that several features affect the WHC of meat from  $RN^-$  carriers.

The higher glycogen content in  $RN^-$  carriers *per se* is also discussed as a possible contributor in combination with the lower protein content in muscle from  $RN^-$  carriers (Monin *et al.*, 1987b; Fernandez *et al.*, 1991)(**Paper II**). Greenleaf *et al.* (1969; Olsson & Saltin, 1970) estimated the capacity of glycogen to bind water in the range of 2-4 times its own weight. This is not a very exact estimation, but the capacity of glycogen to bind water is believed to be large enough to influence the water-holding properties of meat from  $RN^-$  carriers. In fresh meat the glycogen can hold water and thereby partly compensate for the lower protein content. During heating glycogen is broken down into glucose intermediates (**Paper VI**); thus, the glycogen held water is “freed” and expelled. In addition, as mentioned before, the muscle proteins in  $RN^-$  carriers are suggested to denature more easily upon heating (Deng *et al.*, 2002; Bertram *et al.*, 2004). Despite this, meat from  $RN^-$  carriers generally experienced is as juicier than non-carriers (Jonsäll *et al.*, 2000; Jonsäll *et al.*, 2002; Josell *et al.*, 2003a; Josell *et al.*, 2003c)(**Paper II**). Thus, it could be suspected that it is the higher water content found in fresh meat from  $RN^-$  carriers (Monin *et al.*, 1992; Enfält *et al.*, 1997a) that causes the superior juiciness;

in this instance, contradictorily, the water is said to be “freed” during heating. Moreover, the water content in the processed meat is not found to be higher in  $RN^-$  carriers than non-carriers (**Paper I & VI**). It should, however, be remembered that there were no large differences in juiciness between  $RN$  genotypes in **Paper I**. If we hypothesize a relationship between water content in the final product and perceived juiciness, there has to be glycogen left in the cooked meat from  $RN^-$  carriers compensating for the reduced ability of the meat proteins to hold water. In **Paper VI** it was shown that about 19% of the glycogen remained in the cooked meat of  $RN^-$  carriers, whereas non-carriers had no glycogen left as early as 24 h *post mortem*. There was also significantly lower water content in the cooked meat from  $RN^-$  carriers compared with non-carriers, not noted in the fresh meat. Johansson *et al.* (2002) reported a higher water content in raw loins, which was absent when the meat was cooked. Further, Fjelkner-Modig and Tornberg (1986) reported loins of Hampshire origin cooked to 68 °C to be slightly juicier, but to contain less water compared with cooked loin from Yorkshire pigs. Thus, the remaining glycogen after cooking cannot completely compensate for the proteins’ reduced capacity of holding water in  $RN^-$  carriers. Consequently, water content in the raw material cannot alone explain the higher juiciness in  $RN^-$  carriers.

Despite the superior eating quality expressed as tenderness and juiciness of processed meat from  $RN^-$  carriers and tumbled loins found in the present thesis (**Paper II & III**), consumer preferred cured-smoked loins from non-carriers in T1 and ranked non-tumbled loins as more liked in T2. This indicates the importance of combining descriptive sensory analyses with consumer tests. Consumer preference tests assess the personal preference or acceptance of a product (ISO, 1983), while descriptive tests are objective measures on whether samples differ and how much (ISO, 1985a). Thus, the trained panel is not giving their opinion of liking.

Results from descriptive sensory analyses and consumer tests are not always easy to compare. Even though meat from  $RN^-$  carriers scored higher for the important sensory attribute tenderness, the untrained test persons preferred meat from non-carriers (**Paper II**). Tumbled meat was also judged more tender by the trained sensory panel, but non-tumbled cured-smoked loins were generally ranked higher than tumbled cured-smoked loins in the consumer test (**Paper III**). This is in agreement with the findings by Lundström *et al.* (1998b) who found a lower preference for cured-cooked hams from  $RN^-$  carriers than from non-carriers. The conclusion can therefore be drawn that tenderness is of subordinate importance compared with other sensory attributes in this kind of processed meat. This was further confirmed in **Paper III** where the consumers were asked to describe what sensory attributes made them favour the most liked sample. Less than half of the test persons mentioned tenderness, whereas tasty, juicy and right saltiness were mentioned by more than 60% of the test persons.

Unappealing pores are often observed in processed pork. The reason for the formation of unappealing pores in processed meat is not understood yet, even if some attempts have been made to explain the origin. Brauer (1992) tested the effects of pumping pressures, tumbling conditions and microbes on pore formation, but found that none of these factors caused the pores. One theory of the origin of pores, worthy of further study, is the possibility of gas formation during heating of

substances soluble in the meat or meat fluid at low temperatures. Rapid generation of gas during heating, with no possibilities for the gas to escape at the same rate as it is produced, causes bursts in the weakest parts of the structure. Meat stored in CO<sub>2</sub> atmosphere has been shown to develop pores during cooking due to a rapid development of CO<sub>2</sub> (Bruce *et al.*, 1996). The dominating stunning method in Sweden today is lowering pigs in CO<sub>2</sub> and it is hypothesized the CO<sub>2</sub> could accumulate in the carcass and later cause pore formation during cooking, but pores have been found in other species such as beef (author's observation), that are not stunned with CO<sub>2</sub>. However, CO<sub>2</sub> stunning may contribute to an increased number of pores developed. If the above theory of gas formation is true, the ultrastructure of the meat influence pore development. Neither tumbling nor *RN* genotype caused pore formation (**Paper V**), but both caused alterations in the ultrastructure (Cassidy *et al.*, 1978; Estrade, M. *et al.*, 1993a) and thus influenced the frequency of pore formation (**Paper V**). The presence of pores is well known in processed meat, but pores have also been registered in uncured cooked samples (Bejerholm & Aaslyng, 2004). This indicates that the brine is not the direct origin of the pores; however, there may be substances soluble in the brine at low temperatures, which easily transform to gas form during heating. Once formed, the pores probably accumulate potential purge, which is supported by the strong relations between number of pores and relative NMR transverse relaxation population sizes, especially the slowest relative *T*<sub>2</sub> relaxation population, reflecting the more loosely bound water in the meat (**Paper IV**).

Results regarding tumbling are often inconsistent. As an example, intermittent tumbling is shown to have greater impact on structural disruption compared with continuous tumbling (Cassidy *et al.*, 1978), but no differences in tenderness, juiciness and flavour were detected in hams either intermittently or continuously tumbled (Motycka & Bechtel, 1983). Divergent results most likely depend on differences in machinery, processing programs *etc.* Even two products that seem to be similar can differ in final quality due to small differences in the processing (**Paper I & II**). This indicates the importance of the optimisation of the process, which depends on the assembly of machinery used. For example, the difference between tumbling and massaging/mixing is the free falling of the meat. Tumblers are equipped with baffles allowing the meat to drop; meat is recommended to drop about 1 metre to obtain maximum benefit (Treharne, 1971; Pearson & Gillett, 1996). In T1, loins were tumbled in a large commercial tumbler allowing the meat to drop properly, whereas in T2, small experimental tumblers were used permitting only a short fall of loins (see Figure 4). Thus, the mechanical treatment was stronger in T1, which may have contributed to technological and sensory quality differences reported between T1 and T2 (**Paper I, II, III**).

Recently a second mutant allele was discovered at the *PRKAG3* (*RN*) locus (Milan *et al.*, 2000), which here is referred to as the *rn\**. It was found not only to be associated with the Hampshire breed, as for the *RN*<sup>-</sup> allele, but was also identified in Large White, Landrace, Berkshire, Duroc and Wild boar (Milan *et al.*, 2000; Ciobanu *et al.*, 2001). Ciobanu *et al.* (2001) reported that the *rn\** allele affect the meat quality. Lindahl *et al.* (2003) found the *RN*<sup>-</sup> allele to be present to 81% in various crosses of Hampshire and Finnish Landrace, whereof the *RN*<sup>-</sup>/*rn\** genotype was the most frequent. Both the *rn*<sup>+</sup>/*rn\** and *rn*<sup>+</sup>/*rn*<sup>+</sup> were present to 8-

9% and  $rn^*/rn^*$  to 2%. No differences between  $rn^+/rn^*$  and  $rn^*/rn^*$  were found, possibly due to the low frequency of  $rn^*/rn^*$  present. The  $RN^-$  allele dominated over the other two alleles resulting in inferior WHC and lower pH. The  $rn^*$  allele in non-carriers gave higher pH in the loin compared to  $rn^+$  and  $RN^-$  allele. Glycogen content was lower in meat from  $RN^-/rn^*$  genotypes compared to other  $RN^-/-$  combinations, but still higher than in the recessive alleles. Regarding sensory parameters,  $rn^*$  resembled that of  $rn^+$  (Josell *et al.*, 2003a). No classification of  $rn^*$  genotype was undertaken in the studies in the present thesis (**Paper I, II, III, IV, V**). The small differences in meat quality between  $rn^+/rn^+$ ,  $rn^+/rn^*$  and  $rn^*/rn^*$ , and the dominance of the  $RN^-$  alleles over the other alleles presented by Lindahl *et al.* (2003) and Josell *et al.* (2003a), indicate that the absent information of  $rn^*$  genotype does not affect the overall conclusions in the present thesis.

Multivariate statistics have gained ground the last two decades and is commonly used in, for example, sensory science. It is a tool that approaches the interpretation of data in another way and is an excellent complement to conventional statistics (Næs *et al.*, 1996). However, confusion may easily arise when talking about correlations, because in multivariate and conventional statistics the term has different meanings. Ordinary Pearson correlation coefficient reflects the link between two variables in a data set, whereas loading and correlation loading plots of multivariate statistics illustrate the relationship between two variables, in relation to all variables included in the statistical model. Hence, the relationship may not be logical other than in the specific situation given, but give valuable overall information. Consequently, when comparing correlations and relationships the differences between the two concepts have to be kept in mind.

Correlations are often presented across the whole material, but rarely for main effects. This can cause misinterpretation since overall correlations may be a result of reciprocal conditions between main effects and thus not reflect existing correlations. Meat from different  $RN$  genotypes is a good example because there are often large differences in the correlation coefficients both within and across  $RN$  genotypes (Lundström *et al.*, 1998b; Gariépy *et al.*, 1999) (**Paper I**). Hence, it is of major importance to plot the correlations to get an appropriate overview before drawing conclusions. This can probably explain differences in correlations between surveys.

## Conclusions

- Meat from  $RN^-$  carriers had inferior water-holding properties compared with non-carriers. Including tumbling in the process increased the technological yield, but tumbling could not overcome differences in processing yield between  $RN$  genotypes.
- Generally, the presence of the  $RN^-$  allele seems to strongly affect sensory perception. Despite the lower water-holding properties of fresh meat from  $RN^-$  carriers compared with non-carriers, processed meat from  $RN^-$  carriers was assessed as more tender, juicy and acidulous. Although this superior eating quality in terms of tenderness and juiciness according to the trained sensory panel in general, consumers preferred cured-smoked loins from non-carriers of the  $RN^-$  allele.
- Tumbling resulted in superior tenderness, but seemed to only slightly affect juiciness. However, it should be noted that consumers tended to rank non-tumbled loins as most liked.
- Intra-myofibrillar water, determined by LF-NMR  $T_2$  relaxometry related to the sensory attributes juiciness, acidulous taste and meat taste in cured-smoked loins. Generally, water located in the highly organised myofibrillar lattice was of greatest importance for the eating quality.
- The number of unappealing pores developed during processing could be predicted with high precision by relative LF-NMR  $T_2$  population sizes. The close relationship between pore number and the slowest relative  $T_2$  population most likely originated from loose water trapped in the pores.
- Image analysis is a useful, accurate tool when determining degree of pores formed during processing. The ultrastructure of the meat plays an important role in the ease with which pores are formed, but the structure *per se* is not the origin of the pores.
- All glycogen was broken down in non-carriers of the  $RN^-$  allele during the *post mortem* glycolysis, whereas a considerable amount was left in  $RN^-$  carriers. Cooking further broke down glycogen, but intact glycogen was to be found in both the cooked meat and the fluid lost during cooking.
- For an optimum product quality the process design has to be optimised for each step in processing, because even small changes in the process can greatly affect the final product quality. These effects have to be kept in mind when drawing conclusions from and designing studies.

## Future perspectives

- To be able to offer the consumers an optimal eating experience of meat and meat products, understanding of the relationships between the water in the meat and perceived juiciness and other sensory attributes are needed. The methods of NMR combined with sensory analyses needs to be further investigated to increase our knowledge on the complexity of experienced juiciness of meat and its water-binding properties *etc.* Additionally, NMR provides information on ultrastructures in the meat valuable in the understanding of texture parameters.
- There are requirements to clarify the origin of unwanted pore formation. Data tomography and nuclear magnetic imaging (MRI) could provide information about spatial distribution of pores and pore development, and thereby give valuable information on the underlying mechanism of the causes of pore formation. The effect of CO<sub>2</sub> stunning on the degree of pore formation is also a matter for further investigations.
- The exact mechanism behind the lower water-holding capacity of meat from  $RN^-$  carriers is not known. More detailed studies on glycogen and its mechanism regarding water-holding in meat, together with the possible roles of macro- and proglycogen could shed light on the causes of inferior water holding of  $RN^-$  carriers.
- Possible relationships between water content in cooked/processed meat of different  $RN$  genotypes and sensory attributes, such as juiciness need to be further evaluated.

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## Populärvetenskaplig sammanfattning

Köttkvalitet är ett begrepp som innefattar många olika aspekter. Förutom de nutritionella, hygieniska och toxikologiska aspekterna finns ytterligare två viktiga aspekter, ätkvalitet och funktionalitet, vilka behandlas i denna doktorsavhandling. För konsumenten är de tre förstnämnda aspekterna något förutsätts vara av hög kvalitet och därmed väger det inte in lika tungt vid val av kött eller köttprodukter i butik. Den sensoriska kvaliteten eller något förenklat ätkvaliteten är dock mer påtaglig för konsumenten. Valet av kött i butik beror av det visuella intrycket och tidigare erfarenheter. Vi vill att köttet ska se smakligt ut och att det ska vara mört och saftigt. Köttindustrin däremot värderar de funktionella aspekterna högre. Ett kött som tappar mycket vätska under processning eller lagring är en dålig investering eftersom kött betalas efter vikt. Tyvärr går inte alltid konsumentens och industrins kvalitetskrav hand i hand. Ett bra exempel på detta är den s.k.  $RN^-$ -genen.

Under 70-talet började lantbrukskooperationen att använda Hampshire som faderras till våra slaktsvin i Sverige. Men med rasen kom en dominant gen med stora effekter på köttkvaliteten, den så kallade  $RN^-$ -genen. Den visade sig snart vara orsaken till det avsevärt försämrade processutbytet hos kött, vilket resulterar i stora ekonomiska förluster för köttindustrin. Å andra sidan, bedömdes färskt kött med denna gen att oftast vara både mörare, saftigare och även syrligare. Den största andelen kött som konsumeras är dock processat i någon form, men få studier är gjorda på  $RN^-$ -genens effekt på ätkvaliteten hos processade produkter. I ett första steg studerades därför hur  $RN^-$ -genen påverkade den sensoriska upplevelsen och den teknologiska köttkvaliteten hos kassler (rimmad, rökt och värmebehandlad kotlett) (**Papper I & II**). Utbytet visade sig vara 2,7 procentenheter lägre hos kassler med  $RN^-$ -genen än utan den. Den största vätskeförlusten skedde under rimmingsfasen och salthalten var lägre i kassler från  $RN^-$ -bärare ( $RN^-/rn^+$ ) än icke-bärare ( $rn^+/rn^+$ ). Köttet från  $RN^-$ -bärare bedömdes av en tränade smakpanelen som mörare, saftigare och syrligare. Dessutom var kassler från  $RN^-$ -bärare mindre salt i smaken, hade en jämnare rimmad färg och tenderade att innehålla fler oönskade hål och porer. Trots de uppmätta positiva egenskaperna hos kassler med  $RN^-$ -genen föredrog otränade personer i ett konsumenttest kassler utan denna  $RN^-$ -gen (68%). Orsaken till detta är inte fastlagd, men kan vara att kasslern i försöket uppvisade en extrem mörhet, på gränsen till leverlik ha berott på den extrema mörhet kasslern i försöket uppvisade, på gränsen till leverlik. Som konsument har man en bestämd uppfattning om hur kassler ska smaka och den möra kasslern uppfyllde troligen inte konsumentens förväntningar, eller så dominerade andra smakegenskaper över mörheten.

Inte bara gener utan även yttre omständigheter påverkar kvaliteten på de köttprodukter vi äter. Designen på processen vid vidareförädling av kött påverkar i hög grad slutprodukten. Den dominerande processningen av helt kött sker genom att först tillsätta en saltlake för att därigenom öka hållbarhet, förbättra smaken och frigöra saltlösliga proteiner. Därefter röks och värmebehandlas köttet för att få sin slutgiltiga karaktär. Ofta inkluderas även ett tumlingssteg direkt efter injiceringen av salt. Det är en mekanisk bearbetning där köttet roteras i stora rostfria trummor

med skovlar så att friktion och fritt fall uppstår. Denna massage förändrar köttets struktur, vilket möjliggör en snabbare fördelning av saltlake i köttet. Effekterna av saltet och tumlingen ökar köttets förmåga att hålla vatten, vilket är positivt. Hur ätegenskaperna påverkas av tumling är dock inte lika väl dokumenterat som effekterna på de funktionella egenskaperna. Söndringen av cellstrukturen under tumling anses dock gynna både mörheten och saftigheten, men de flesta gjorda studier jämför olika tumlingsstrategier och inte effekten av att tumla köttet eller ej. Därför undersöktes effekterna av tumling och *RN*-genotyp på den sensoriska upplevelsen och de teknologiska egenskaperna hos kassler (**Papper I & III**). Högerkotletten från grisar av båda *RN*-genotyperna tumlades under processningen, medan vänsterkotletterna fick vila. Kassler från *RN*-bärare hade ett 4,8 procentenheter lägre utbyte jämfört med kassler från icke-bärare, medan tumling ökade utbytet med 2,4 procentenheter. Sämst utbyte hade med andra ord otumlade *RN*-bärare. Tumling gav också ett mörare kött med lägre syrlighet, betydligt mindre oönskade porer och en jämnare färg. Dessutom tenderade tumlingen att ge en ökad slutlig saftighet. Effekterna av *RN*-genotyp på ätegenskaperna var betydligt mindre än i första studien, men kassler med *RN*-genen bedömdes dock som syrligare och saltare. Vidare tenderade den att vara saftigare, ha ett ökat antal porer och att vara ojämnare i färgen. Inga skillnader i texturgenskaper förelåg. I ett konsumenttest där prover från de olika behandlingarna rangordnades rankades otumlad kassler oftast som godast av ca. 60% av försökspersonerna, vilket återigen talar för att mörhet är av underordnad betydelse jämfört med andra ätegenskaper.

En av de viktigaste egenskaperna för köttkvaliteten är den vattenhållande förmågan (VHF). Hög VHF hos kött ger dock inte självklart ett saftigt kött, vilket *RN*-genen återigen är ett bra exempel på. Trots sin sämre förmåga att hålla vatten, så upplevs detta kött oftast som saftigare. Det är därför troligare att vattnets fördelning och tillgänglighet är viktigare för saftigheten än VHF hos köttet. Ett sätt att mäta vattnets fördelning i köttet är med hjälp av lågfälts-NMR (nuclear magnetic resonance)  $T_2$  relaxometri. Tre olika vattenfraktioner i kött har tidigare uppmätts i färskt kött med denna metod. Först finns det en fraktion med kort relaxations tid som troligtvis motsvarar vattnet tätt sammanlänkat med muskelproteiner ( $T_{2b}$ ). Den mellersta, men klart största fraktionen av vatten, ligger innesluten i myofibrillerna ( $T_{21}$ ) och slutligen den fraktion av vatten vid långa relaxationstider som motsvarar vattnet utanför både myofibrillära nätverket och muskelcellerna, *d.v.s* det mest löst bundna vattnet. För att finna eventuella samband mellan vattnets fördelning i köttet och den sensoriska upplevelsen av köttet studerades tumlad och otumlad kassler från båda *RN*-genotyper med hjälp av LF-NMR och relaterades till ätegenskaperna beskrivna i Papper II (**Papper IV**). NMR  $T_2$  variablerna, och då i huvudsak tidskonstanten  $T_{21}$ , korrelerade till saftighet, syrlig smak och köttsmak. Antalet porer hade ett starkt samband med den inbördes storleken på de tre vattenfraktionerna. Tydliga skillnader i egenskaper mellan de två *RN*-genotyperna förelåg för den största vattenfraktionen. Både tumling och *RN*-genotyp påverkade sambanden mellan NMR  $T_2$  variabler och sensoriska attribut.

Inte alltför sällan uppstår små hål eller porer i processat kött som är synliga för ögat och som ger ett negativt intryck av produkten. Orsaken till dessa oönskade hål är inte fastställd, men effekten som både *RN*-genen och tumling har på

köttstrukturen borde påverka bildningen av dessa porer. Bedömning av porbildning i kött har hittills gjorts med hjälp av tränad sensorikpanel eller subjektivt, vilket är antingen kostsamt eller för subjektivt. Därför studerades effekterna av  $RN$  genotyp och tumling på porbildningen i kassler med hjälp av både bildanalys och sensorisk panel (**Papper V**). Tumling och  $RN$  genotyp påverkade bildningen av porer, även om effekten var starkare för tumling. Men ingen av effekterna orsakade eller kunde helt motverka porbildningen. Tumling reducerade antalet porer kraftigt, troligtvis beroende på förändringen av strukturen och frigörandet av saltlösliga proteiner under tumlingen. Effekten var också större hos tumlade bärare av  $RN^-$ -genen än hos icke-bärare. Kassler från  $RN^-$ -bärare hade tendens till mer porer än kassler utan genen, vilket troligtvis kan förklaras med den något lösare struktur som påvisats hos bärare av  $RN^-$ -genen. Korrelationerna mellan de sensoriska/subjektiva bedömningarna och bildanalysresultaten var mycket höga ( $r=0,80-0,90$ ), vilket kan tolkas som att bildanalys kan anses vara ett exakt, snabbt och objektivt sätt att bedöma förekomsten av t.ex. porer.

Det som är unikt för  $RN^-$ -genen är att den ger ett ca 70% högre glykogeninnehåll i glykolytiska muskler samtidigt som musklerna innehåller mindre andel protein och mer vatten. Det är också detta som är orsaken till den sämre förmågan att hålla vatten hos detta kött. Vattnet hålls till största delen av muskelproteinerna och med en mindre andel protein minskar  $RN^-$ -bärarnas förmåga att hålla vatten. Glykogen binder dock till sig vatten i motsvarande mängd som protein (~3 ggr sin vikt), vilket kompenserar den mindre mängd vatten som kan hållas av proteiner i kött från  $RN^-$ -bärare. Under uppvärmning anses glykogenet frigöras från vattnet som det har hållit bundet, vilket skulle förklara den större vätskeförlusten hos  $RN^-$ -bärare under uppvärmning. Mekanismen bakom denna frigörelse är inte närmare studerad och i ett pilotförsök studerades koncentrationerna av glykogen och dess nedbrytningsprodukter i färskt kött, rå köttsaft, kokt kött och köttspad hos bärare och icke-bärare av  $RN^-$ -genen (**Papper VI**). Glykogenet hade redan 24 timmar efter slakt helt brutits ned i kött från icke-bärare, medan en betydande andel fanns kvar i kött från  $RN^-$ -bärare. Under kokning bröts en del av detta glykogen ned, men glykogen återfanns både i det kokta köttet och köttspadet. Det visar att glykogenet inte bara släpper ifrån sig vatten under kokning, utan själv läcker ut ur köttet. Vid kokning bryts membran och andra strukturer i köttet troligtvis sönder, vilket möjliggör för glykogenet att läcka ut och därmed bidra till den lägre vattenhållande förmågan hos  $RN^-$ -bärare jämfört med icke-bärare.

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