# INHIBITION OF WARMED-OVER FLAVOUR IN PORK BY ADDED HEATED MEAT JUICE FROM RN⁻ PHENOTYPE OF HAMPSHIRE CROSSBRED PIGS

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## Background

The Maillard reaction is a very important one in many industrial food processes, including the processing of meat. The significance of the Maillard reaction to food is manifold: the production of colour, the production of flavour or off-flavour, the reduction of nutritional value, toxicity and antioxidative properties. The Maillard reaction consists of a very complex network of many different chemical reactions, in which, during the early stages, amino acids combine with sugars. The content of reducing sugars and free amino acids in meat varies with intrinsic factors such as age, species, genotype, muscle type, ageing, condition before slaughter (e.g. stress), and extrinsic factors such as slaughtering and chilling methods. During post-mortem glycolysis, reducing sugars and lactic acid are formed from glycogen. Pork from the Hampshire breed may contain very high levels of reducing sugars. A major dominant gene, called $RN^-$ (Rendement Napole), may be present in pork from the Hampshire breed and crossbreeds. Meat from carriers of the $RN^-$ gene ($RN^-$ phenotype) has a high glycogen content in the muscles, while non-carriers ($rn^+rn^+$ phenotype) have a normal glycogen content (1-3).

An off-flavour associated with lipid oxidation, warmed-over flavour (WOF), is formed during the chill-storage of cooked meat products. Many Maillard reaction products (MRPs) are known to have an antioxidative effect, which may be used to prevent WOF. The measurement of volatiles, such as hexanal, and thiobarbituric acid reactive substances provides good correlation with sensory perceived WOF (4). The inhibition of WOF is an important quality issue enabling the meat processing industry to produce high quality products, which meet exacting consumer preferences.

## Objective

The objective of this investigation was to study whether antioxidative MRPs, inhibiting the formation of WOF during the storage of cooked meat, were formed in heated pork meat juice from two phenotypes ($RN^-$ and $rn^+rn^+$) from Hampshire crossbred pigs.

## Materials and methods

Lyophilized meat juices from pork from Hampshire crossbred pigs of the $RN^-$ and $rn^+rn^+$ phenotypes, respectively, were heated in an autoclave (1 h, 121°C) in order to produce MRPs. The antioxidative effect of the heated meat juices was evaluated in cooked and chill-stored minced pork patties. Fresh pork (*M. bicep femoris*) was ground and standardised to a fat content of 10%. Heated meat juices were added to the minced meat (3% v/w). As a control, water (3% v/w) was added to the meat. Patties (80±1 g) were cooked in an oven at 180°C to a final internal temperature of 80°C. The samples were covered with aluminium foil to avoid browning during heating. The patties were stored at 4°C for 0-2 days in oxygen permeable plastic bags, then vacuum packed, and kept frozen (-80°C) until analysed for chemical composition and lipid oxidation.

The phenotypes ($RN^-$ and $rn^+rn^+$, respectively) were deduced by determining the glycogen levels in the raw meat as the sum of glycogen, glucose and glucose-6-phosphate (5). Animals with a glycogen concentration ≥40 µmole/g meat were regarded as carriers of the $RN^-$ allele. Non-protein nitrogen was analysed in the raw meat, according to Kjeldahl, after precipiting the proteins with sulfosalicylic acid (6). The cooked pork patties were analysed for hexanal and pentanal content, using HS-GC-MS, and TBA-value (2-thiobarbituric acid) before and after 2 days of chill-storage (7).

For the analysis of hexanal and pentanal, pork samples (20 g) were homogenised and equilibrated for 15 min at 25°C in an absorption tube. Volatile compounds were absorbed on a Tenax trap (Tenax TA, 60-80 mesh) by passing helium through the absorption tube for 15 min (flow rate 60 ml $min^{-1}$). The volatile compounds were desorbed at 250°C for 30 min with a helium flow of 60 ml $min^{-1}$ in a Perkin-Elmer ATD400 automatic thermal desorption system and retrapped on a Tenax-packed cold trap maintained at -30°C. The volatile compounds were injected into the GC-column by thermal desorption of the trap at 300°C for 2 min with a split 1:15. A GC 8000 gas chromatograph (Fisons) connected to a Trio-1000 mass spectrometer (VG Masslab) was used for the GC-MS analysis. The following chromatographic conditions were used: HP-1701 capillary column, 0.25 mm x 30 m, film thickness 1.0 µm; oven temperature 50°C for 2 min, 50°C to 200°C with a slope of 5°C $min^{-1}$, 200°C to 220°C with a slope of 20°C $min^{-1}$ and finally 220°C for 6 min; helium flow 1.3 ml $min^{-1}$. Electron impact mass spectra were recorded with an ionisation energy of 70 eV. 1-Chlorononane was used as the internal standard (ISTD).

Analysis of variance (ANOVA), using the model y=constant+phenotype, followed by Tukeys' Pairwise Comparison test, was performed using SYSTAT (Wilkinson, Leland, version 7.0).

## Results and Discussion

The heated meat juice from pork of the $RN^-$ phenotype had a much darker colour than the meat juice from the $rn^+rn^+$ phenotype, indicating that more melanoidines had been formed. The hexanal content was significantly lower in patties with added meat juice from the $RN^-$ phenotype, compared to samples with added meat juice from the $rn^+rn^+$ phenotype or samples without added meat juice (43% reduction) on day 0 (Table 1). After chill-storage for 2 days, the hexanal and pentanal levels and TBA values were significantly lower in the pork patties with added meat juice from $RN^-$ phenotypes, compared to the $rn^+rn^+$ phenotypes or the control.

The relative inhibition in the formation of lipid oxidation products during cold storage of pork patties with added meat juice, compared to control samples without added meat juice, is shown in the Table (Table 1).

Meat juice from the $RN^-$ phenotype had a high glucose content (703 mg/100 g raw meat), while meat juice from the $rn^+rn^+$ phenotype had a lower glucose content (132 mg/100 g raw meat). The strong antioxidative effect of heated meat juice from the $RN^-$ phenotype may be explained by the high glucose content, as no differences in the content of non-protein nitrogen were found (34 mg/100 g raw meat).

The addition of antioxidative MRPs during the production of cooked meat products for chill-storage may be one way of inhibiting the formation of WOF and producing high quality meat products. The effect on the flavour characteristics of pork patties with added meat juice needs to be studied, since no sensory analysis was performed in the present investigation. Moreover, the safety of heated meat juice needs to be further evaluated, due to the possible formation of carcinogenic heterocyclic amines (8).

## Conclusion

The addition of heated meat juice from pork from Hampshire crossbred pigs of the $RN^-$ phenotype to pork patties significantly reduced the formation of lipid oxidation products during cooking and chill-storage, compared to samples without added meat juice or with added meat juice from the $rn^+rn^+$ phenotype. The strong antioxidative effect of heated meat juice from the $RN^-$ phenotype may be explained by the high glucose content of the meat, as no differences in the content of non-protein nitrogen were found.

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**Table 1.** LS-means of the hexanal and pentanal levels (%/ISTD) and TBA values (mg MDA/kg) in cooked chill-stored pork patties with added meat juice. The relative inhibition (%), compared to control samples, is shown.

| | | Hexanal[1] | | Pentanal | | TBA value | |
|---|---|---|---|---|---|---|---|
| **Sample/Storage (days)** | **n** | **0** | **2** | **0** | **2** | **0** | **2** |
| **Control** | 4 | $527^a$ | $660^a$ | $123^a$ | $222^a$ | $1.5^a$ | $4.9^a$ |
| $RN^-$ | 4 | $302^b$ | $398^b$ | $77^a$ | $125^b$ | $0.9^a$ | $2.9^b$ |
| Inhibition (%) | | 43 | 40 | 37 | 43 | 38 | 40 |
| $rn^+rn^+$ | 4 | $469^a$ | $573^a$ | $147^a$ | $202^a$ | $1.6^a$ | $4.8^a$ |
| Inhibition (%) | | 11 | 13 | 0 | 9 | 0 | 3 |

[1]LS-means having a different index within a column differ significantly according to $p \leq 0.05$.