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The formation of water-soluble antioxidants in chicken held at 80°C

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Processing to end-point temperatures of 70 or 80°C in the core region of ground chicken packs in a water bath at 80 ± 1°C, induced measurable differences in the resistance to oxidative deterioration during chilled storage, with the product processed at the higher temperature showing the greater stability. The TBARS development appeared to have been related to the intensity of the heat treatment within the temperature ranges studied. As shown by dialysis, the inhibitory action against oxidation appeared to be related to the production of water-soluble low-molecular-weight compounds formed during the heating process. The arrest of the oxidative changes during refrigerated storage was enhanced by incorporating glucose into the recipe. This indicated that the antioxidant activity developed during processing probably involved the Maillard reaction.

INTRODUCTION

Investigations of the factors promoting oxidative processes in meats have shown that thermal processing catalyses those changes (Younathan & Watts, 1960). In general, the rate of oxidation increases as the temperature is increased. However, the intensity of heat treatment has been shown to both increase (Spanier *et al.*, 1992) and decrease (Goll *et al.*, 1992) the degree of lipid oxidation. There are a number of studies showing the accelerating effect of heating on the development of oxidative rancidity in meats (Younathan & Watts, 1959, 1960; Sato & Hegarty, 1971; Keller & Kinsella, 1973; Williams & Harris, 1983). It is believed, that during heating, changes occur in the muscle pigments which may affect their pro-oxidant activity (Pearson *et al.*, 1977). Also, other researchers have shown that stabilisation against autoxidation by heat treatment occurs during heating (Zipser & Watts, 1961; Sato *et al.*, 1973; Einerson & Reineceius, 1977; Huang & Green, 1978). Yamauchi (1972) reported that the TBA number of cooked meats decreased progressively with increasing temperatures when heated above 80°C. Previous studies suggest that intermediates or products of the Maillard reaction might be important in this context (Lingnert & Eriksson, 1980*a*,*b*, 1981).

The primary objective of this study was to explore further the effect of temperature, within the range of standardised and specified cooking procedures, on the development of oxidative changes in cooked ground chicken thigh meat during refrigerated storage. The secondary objective was to develop a methodology for studying the nature of the antioxidant activity observed in chicken cooked to core temperature of 80°C. In this study WOF was quantified by measuring thiobarbituric acid reactive substances (TBARS).

MATERIALS AND METHODS

Source of meat

Fresh chicken thighs were purchased from a local wholesaler approximately 24 h *post mortem*. According to the wholesaler, the birds were fed fish meal and between slaughter and purchase they were kept refrigerated.

Immediately after delivery the thighs were wrapped individually in aluminium foil, blast frozen at −40°C, and stored at −30°C until required for processing prior to assay.

Product preparation

When required, an appropriate number of thighs was randomly selected and defrosted by storing at +5°C for 24 h, washed with tap water and deboned manually. The skin, all visible external fat, and connective tissue were removed.

The meat was ground in a 'Robot-Coupe' food processor equipped with a plastic work bowl and a stainless-steel blade for 1 min to obtain a mince-like product. When glucose was included in the recipe formulation, care was taken to ensure the product was thoroughly mixed. (Average TBA value of the prepared raw mince was 0·5 ± 0·1 mg MDA/kg meat.)

Containers and conditions of cooking

The homogenised meat (126 ± 1 g) was filled into lacquered cans with inner dimensions 65·4 × 50·5 mm and wall thickness 0·165 mm. The cans were vacuum-sealed and immersed in a water bath set at 80 ± 1°C equipped with a 'Haake E8' water circulator. Plastic insulating spheres were used as a floating lid for the water bath, to minimise evaporation and heat losses.

The core region temperature of the food was monitored by insulated copper–constantan wire thermocouples of 0·19 mm diameter, type T, positioned at the geometrical centre of a can.

The thermocouple was inserted into the can using a brass gland initially designed for use with pouches. For a perfect seal a silicone rubber washer with a perforation of the thermocouple diameter was fitted between the brass gland and the inner wall of the can. The thermocouple tip was carefully mounted in the geometrical centre of the can during filling. Keeping the thermocouple in place was not troublesome because the food material was in the form of a thick paste.

Process control equipment

The thermocouples were connected to a 'Comark 5335' scanning thermometer linked to a computer programmed for the calculation of the centre 'cook-value' achieved at several time intervals. The water bath temperature was continuously monitored by a thermocouple immersed in the heating medium.

Cooling

The cans were removed when the core region reached an end-point temperature of either 70 or 80°C and cooled under running tap water for approximately 30 min (internal temperature of 15–20°C).

Sampling

All possible necessary precautions were taken to prevent adventitious autoxidation during sampling. After cooling was completed, the cooked contents of those cans bearing the same composition were blended again in a food processor for 1 min. The objective was to reduce sample variability resulting from small differences due to water bath position.

Subsamples (10 g) were weighed into resealable polythene sampling bags with dimensions 89 × 114 mm and material thickness 0·16 mm. Sampling was completed within 1·5 h after cooling.

Chilled storage of cooked chicken

The chilled storage was accomplished in a cold room at +5°C for up to 6 days including the day of cooking. The air in the cold room was circulating at an average velocity 0·4 m/s as measured by a vane anemometer.

The preweighed subsamples of the cooked and remixed paste-like chicken sealed in polythene bags were shaped into a thin layer, which approximated the shape of a slab with an average pack thickness of 3 mm. The packs were labelled for their composition and required storage time and randomly distributed in a single layer on trays.

Samples were frozen to stop biochemical reactions until they were required for testing for TBA value.

TBARS

The progress of cooked chicken autoxidation during storage was followed using TBA determinations. The modified distillation method by Tarladgis *et al.* (1964) was used for the determination of TBARS levels with a further modification described by Pikul *et al.* (1983). TBA values were expressed as mg of malondialdehyde (MDA) per kg of chicken sample.

Distillation apparatus

A Buchi 315 nitrogen steam still, originally designed for protein determination, was used for the distillation of TBARS from chicken. This was a compact device equipped with an electrode steam generator (rated output: approximately 2 kg steam/h) which was connected to a distillation unit, operating with a distillation rate of 25 ml/min and 250 ml of distillate were collected. Karastogiannidou and Ryley (1994) reported that these conditions gave 88% recovery of malondialdehyde from chicken.

Dialysis

Sample preparation

A 10 g sample of freshly cooked and mechanically homogenised chicken was placed in a dialysis tube (Gallenkamp) with 20 ml distilled water. The molecular weight cut-off of this material was in the range 12 000–14 000 and its inflated diameter 78 mm. The tubing was closed at both ends by three successive knots and immediately immersed in a 5 litre glass beaker containing distilled water and a magnetic stirrer and held in place by a clamp. Dialysis was allowed to proceed in a cold room (+5°C) for 24 h. The water was changed once and the dialysate was rejected.

Sample composition

Glucose (BDH) at 1·5 % level (w/w) was incorporated in all chicken samples prior to cooking with the aim of enhancing the Maillard reaction and to facilitate a more distinct measurable effect of the dialysis.

Controls

Two controls were adopted to monitor the dialysis experiments. These were as follows:

(a) 10 g meat with 20 ml water stored in dialysis tubing, analogous to the dialysis samples; however, the controls were wrapped in foil before immersion in water to prevent dialysis; and

(b) 10 g meat stored in polythene bag, in a refrigerator at +5°C.

Storage conditions

After the dialysis was effected (24 h), the test samples and controls were removed from the dialysis bath and stored at +5°C in a desiccator under an atmosphere saturated with moisture, so that any moisture loss was avoided. The storage time was up to 6 days and was followed by the TBA test.

The TBA value of the sample at the end-point of the dialysis is compared to that on the first day of storage because, as long as the dialysis was proceeding, the oxidative reaction was expected to occur simultaneously. Both test samples and controls were analysed in triplicate.

RESULTS AND DISCUSSION

Oxidative stability of chicken cooked to 70 and 80°C centre temperature

Heat penetration curves (Fig. 1) revealed that cooking at a controlled heating medium temperature of 80 ± 1°C required less than 40 min and less than 60 min for the geometrical centre of the cans to reach 70 and 80°C, respectively.

Mean cook-values of chicken processed until the centre temperature reached 70 or 80°C were 1·3 min (1·2–1·4) and 4·4 min (4·2–4·6), respectively. The cook-values calculations were based on a *z*-value of 25°C and a reference temperature of 100°C.

The level of TBARS at all storage times was dependent on the cooking procedure. Samples cooked until the core region reached 70°C showed higher TBA numbers after storage than samples cooked until the temperature in the centre reached 80°C (Fig. 2). The longer process under those conditions was enough to lower the TBA values of cooked chicken during storage by 50% in the critical initial storage period. TBA numbers on the second day of refrigerated storage for control samples cooked to 70°C centre temperature, approached those obtained on both the fourth and fifth day of storage for controls when the heat treatment was effected to 80°C core temperature (Fig. 2). This is an indication that inhibitors of formation of the TBARS might have been produced during cooking. It is recognised that the Maillard reaction may influence the oxidative stability of food products, since some of the reaction products have the ability to retard lipid oxidation (Pearson & Gray, 1983).

The heat treatment applied during cooking in the present study was far below the retort temperatures mainly reported in the literature in connection with browning. The 80°C centre temperature has been reported as a threshold cooking temperature for lowering the TBA values of cooked meats (Yamauchi, 1972). The chicken samples cooked to a centre temperature of 80°C, which was also the heating medium temperature, did not appear to have a visible difference in colour intensity from those samples cooked to 70°C centre temperature. However, the samples cooked to 80°C centre temperature had their outer surface exposed for a longer period at the cooking temperature (water bath temperature was set at 80°C) than those samples cooked to 70°C.

In conclusion, the 80°C centre temperature heat treatment may have promoted the formation of compounds—probably Maillard products—which contributed to the antioxidative effect observed during storage (Eichner, 1981).

Oxidative stability of samples cooked to 70 and 80°C centre temperature and mixed prior to storage

Two cooking processes were carried out (70 and 80°C centre temperature) and, following cooling, the two batches were combined, mixed and stored. The TBA values of this mixture are presented as 'experimental' in Fig. 2 and they are means of duplicate samples. The latter were compared with the calculated mean TBA values of chicken samples cooked to a centre temperature of 70 and 80°C for each storage day. These are quoted as 'calculated' and accordingly, they are means of duplicate samples from each of two cans (i.e. four TBA values).

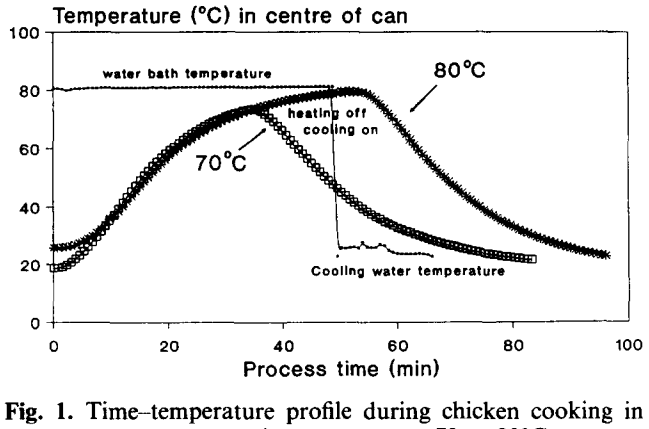

Fig. 1. Time–temperature profile during chicken cooking in a can to core region temperature 70 or 80°C.

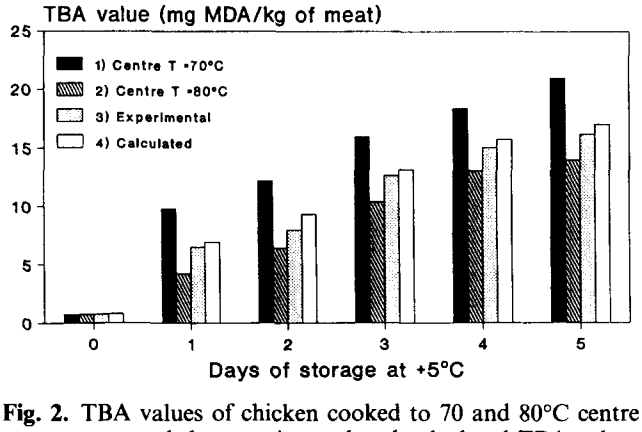

Fig. 2. TBA values of chicken cooked to 70 and 80°C centre temperature and the experimental and calculated TBA values of a mixture during 5 days chilled storage.

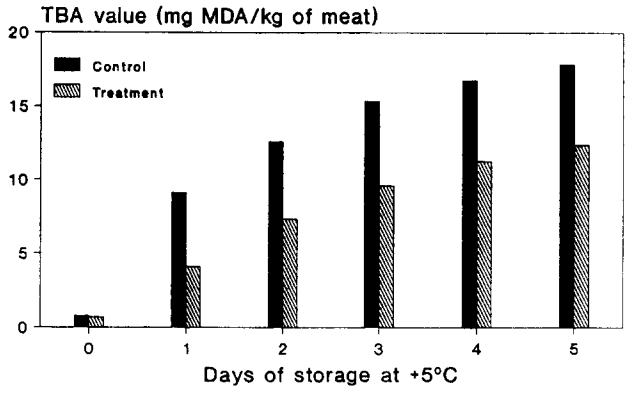

Fig. 3. TBA values of chicken cooked with and without 1·5% glucose during chilled storage.

As indicated in Fig. 2, mixing and combined storage of the two batches was shown to have promoted lower TBA numbers than the calculated ones during chilled storage ($P < 0.05$ by Students' t-test).

These findings suggest that the antioxidant compounds formed in the chicken cooked to 80°C in the centre were able to arrest the oxidation of the whole mixture, indicating mobility in the system.

Effects of glucose as a part of the recipe formulation

Since the general route for the Maillard reaction is the condensation of sugars with amino acids, it would be expected that increasing the concentration of reducing sugars present in the system would increase the reaction rate. Addition of 1·5% glucose to the chicken prior to cooking enhanced the antioxidative activity as measured by the TBA test during 5 days of storage at +5°C (Fig. 3).

Significant ($P < 0.05$ by Students' t-test) differences were found on the first, second and fifth storage day between TBA value means of samples cooked together with glucose and samples cooked without the sugar. The difference on the third and fourth day marginally approached the 0·05% level of probability, while it was significant at the 0·1% level.

These results suggest that glucose, as a part of the recipe formulation, aids the oxidative stability of cooked chicken. This indicates the occurrence of a reaction requiring a Maillard reactant during the heat processing of the food.

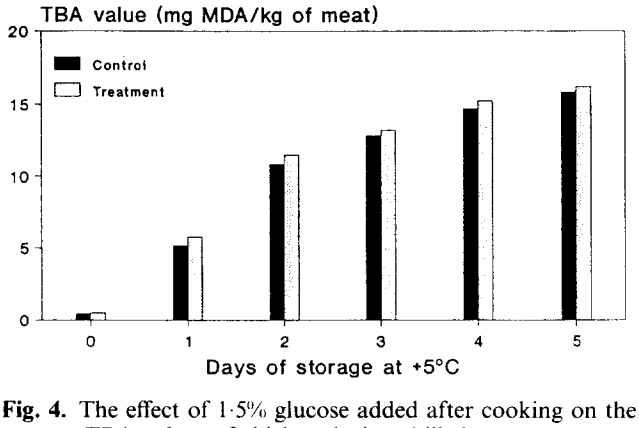

Fig. 4. The effect of 1·5% glucose added after cooking on the TBA values of chicken during chilled storage.

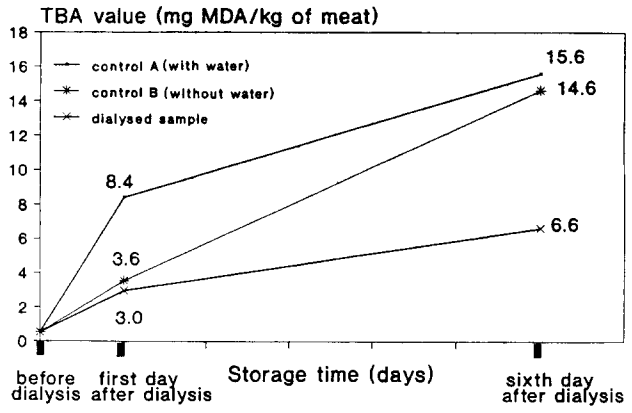

Fig. 5. The effect of dialysis prior to storage on the TBA values of cooked chicken during chilled storage.

Nonetheless, verification that the heating of glucose produced the antioxidant activity rather than the presence of the sugar itself, was felt to be necessary because of the scavenging properties towards free radicals that the sugars possess due to their hydroxyl groups. The same amount of glucose, therefore, was added to the food system after cooking so that contact was limited to the storage step only. TBA values increased with the glucose application after cooking was completed (Fig. 4). The increase in TBARS, as glucose was applied after heat treatment, might be a result of microbial-enzyme-induced oxidation promoted by the sugar.

Thus, these findings indicate that it is the cooking conditions of the food system that appear to promote the formation of compounds with antioxidative properties.

Separation of the antioxidants from the food matrix with respect to their molecular size by dialysis

Dialysis alone followed by storage at +5°C was found to be unsuitable for indicating the presence of water-soluble antioxidants. MDA, being water-soluble, was lost by leaching, so that the expected increase in TBA value was not obtained. In addition, the presence of water was shown to have a pro-oxidant effect initially (Fig. 5). Therefore, further experiments were designed, which involved back-addition of dialysed chicken to the freshly cooked samples.

Glucose was included in the recipe formulation for enhancing the antioxidative activity, thus boosting any difference between controls and test samples after storage. The results are outlined in Table 1. As can be seen, the back-addition of a dialysed sample to a freshly cooked sample induced a higher TBA value (11·6) after 3 days of storage than the back-addition of a non-dialysed sample (7·6). Since it was previously shown (Fig. 5) that, during dialysis, some MDA loss was taking place, the value of 11·6 was unexpectedly high. Thus, it can be concluded that antioxidant materials were washed out by dialysis.

Since the antioxidative compounds generated during the heat treatment were shown to be dialysable, they were consequently low-molecular-weight substances.

Table 1. TBA values of cooked chicken after 3 days of storage at +5°C, which was subjected to the back-addition of dialysed and non-dialysed chicken

Time (days)	TBA value (mg MDA/kg chicken)[a]	
	10 g Dialysed[b] added to 10 g freshly cooked	10 g Non-dialysed[c] added to 10 g freshly cooked
3 days after back-addition	11·6 (0·1)	7·6 (0·3)

[a] TBA values are means of two analytical replications (i.e. two dialyses); each dialysed sample was a distillation test-sample. The range of each mean is presented in parentheses.
[b] Dialysed: cooked chicken with 20 ml water subjected to dialysis. (Oxygen access was facilitated via dialysing water.)
[c] Non-dialysed: cooked chicken with 20 ml water stored in a polythene bag which was wrapped in foil and maintained at dialysis temperature. This sample served as the control sample in the dialysis experiments. (Limited oxygen due to water and packaging.)

There is strong support in the literature for the concept that low-molecular-weight non-browned reaction products offer protection toward warmed-over flavour formation in meats as measured by the TBA test (Bailey *et al.*, 1987). Various mechanisms have been suggested for the antioxidative activity of Maillard reactants. These include free radical stabilisation via interaction of the free radicals formed during the heating of sugars and amines with the free radicals formed in the lipid oxidation, as well as via complexation of metals including iron, which might catalyse lipid oxidation to form undesirable flavour compounds (Lingnert *et al.*, 1983). Eichner (1981), proposed that, in the presence of Maillard reaction intermediates, the oxidative stability of fatty acids is increased. He also ascribed this increase to inactivation of the hydroperoxides formed by lipid oxidation to products which are unable to form free radicals and rancid products.

CONCLUSIONS

In this study, precise control over the applied heat treatment, has revealed that variations in the cooking practice induced measurable differences in the resistance to oxidative deterioration during chilled storage. The employed cooking scheme ranged within the bounds of normal cooking practices for low-temperature processes.

This resistance was shown to be related to the production of water-soluble low-molecular-weight compounds. The resistance was enhanced by the inclusion of glucose in the recipe, thus supporting the concept that development of Maillard compounds occurred during the heating process and inhibited oxidative changes.

Overall, it was shown that lipid oxidation inhibition capacity can be introduced into cook–chill foods by the proper use of process parameters.

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