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The Influence of Additives on the Oxidation of Pork Back Fat and its Effect on Water and Fat Binding in Finely Comminuted Batters

I. Bloukas* & K. O. Honikel

Federal Centre for Meat Research, Kulmbach, FRG

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ABSTRACT

We have found in previous experiments that slaughter-fresh processed pork back fat enhances batter stability in frankfurter-type sausages. In an attempt to clarify whether the decrease of batter stability with storage time of the fatty tissue is due to the fat oxidation a series of experiments was conducted using sodium nitrite and nitrate, 6-O-palmitoyl-L-ascorbic acid and DL-α-tocopherol as additives to the fatty tissue.

It was found that sodium nitrite and nitrate, although the first is regarded as an antioxidant in meat, had a pro-oxidant effect when added to the fatty tissue. The palmitoyl-L-ascorbic acid showed the best antioxidative activity, and the DL-α-tocopherol was less effective as an antioxidant.

Contrary to its effect on the increase in fat oxidation, sodium nitrite addition gave a remarkable improvement in batter stability, expressed as jelly and fat separation, which was more pronounced on the second day of storage of the fatty tissue.

The pro-oxidant effect of nitrite increased up to a concentration of 375 ppm added to the fatty tissue. Concentrations of nitrite in fatty tissue up to 125 ppm had no influence on jelly release but at higher concentrations the jelly separation decreased. All nitrite concentrations, from 62·5 to 2250 ppm, showed significantly lower fat separation than for the control treatment, especially on the eighth and 15th day of storage of the fatty tissue.

The results show that fat oxidation has little effect on batter stability. Further experiments are needed to clarify the mode of action of nitrite in improving batter stability.

* Present address: Aristotelian University of Thessaloniki, Faculty of Agriculture, Department of Food Science and Technology, GR-54006 Thessaloniki, Greece.

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INTRODUCTION

In most of the meat products manufactured in Western societies pork fat is used as the fat source. The fat content of meat products varies widely, from less than 5% to 55–60%. Also, the shelf-life varies between a few days and more than 6 months. The longer shelf-life is necessary in traditionally produced raw (salami-type) sausage or raw hams (e.g. Parma hams).

The large majority of meat products is stored in air-permeable covers, or may even be kept without packaging. This permits the oxidation of unsaturated fatty acids, which is a problem in raw products. As pork fat contains about 60% unsaturated fatty acids it is interesting to learn how oxidation can be prevented and which additives are effective under which conditions. Nitrite and nitrate, the latter by reduction to nitrite, retard or prevent rancidity in meat (MacDonald *et al.*, 1980; Morrissey & Tichivangana, 1985); ascorbates, citrates and tocopherols are also used for this purpose (Benedict *et al.*, 1975; Dziezak, 1986). However, over the years, various workers (Lea, 1934; Ellis *et al.*, 1968; Coxon *et al.*, 1986; Bloukas & Honikel, in press) have made the contradictory observation that nitrite and nitrate added to pure pork back fat enhance its rancidity on storage. This needed further investigation. In this paper we used finely comminuted meat products as the object of our studies, as we found in other experiments that slaughter-fresh processed pork back fat in frankfurter-type sausages enhances batter stability (Bloukas & Honikel, submitted), and the question arose, if fat oxidation is the reason why batter stability is reduced with storage of fat.

MATERIALS AND METHODS

Preparation of pork back fat samples

Fresh pork back fat was taken from a local slaughterhouse about 1–2 h *post mortem*. After removing the skin, the fat tissue was cut into small pieces, mixed, and minced once in a mincer with a 3-mm mincer plate. The minced fat was divided into equal portions and the following additives were added per kilogram of fat.

Experiment A:
A: No additives (control).
B: 2 g of sodium nitrite ($2{\cdot}9 \times 10^{-2}$ M or 2000 ppm).
C: 2·4 g of sodium nitrate ($2{\cdot}8 \times 10^{-2}$ M or 2400 ppm).
D: 1·5 g of 6-*O*-palmitoyl-L-ascorbic acid ($3{\cdot}7 \times 10^{-3}$ M or 1500 ppm).
E: 2 g of DL-α-tocopherol ($4{\cdot}6 \times 10^{-3}$ M or 2000 ppm).
The experiment was repeated three times.

Experiment B:

A: No addition (control)	or	0	ppm sodium nitrite.
B: 0·0625 g/kg	or	62·5	ppm sodium nitrite.
C: 0·125 g/kg	or	125	ppm sodium nitrite.
D: 0·375 g/kg	or	375	ppm sodium nitrite.
E: 1·125 g/kg	or	1125	ppm sodium nitrite.
F: 2·25 g/kg	or	2250	ppm sodium nitrite.

The experiment was done in duplicate.

After complete mixing of additives with the fat, each portion was divided into four equal samples, each weighing about 480–500 g. The samples were put on plastic trays and were placed in a dark cooler at 0°C and 70–75% RH. Samples from each portion were removed at random between days 0 and 16 of storage and used in batters.

Preparation of meat samples

Lean slaughter-fresh beef (less than 5% fat) was purchased fresh from a local meat market 5–6 days before the start of each series of experiments. At that time, the visible fat and connective tissue were removed, the meat was cut into small pieces (3–8 cm), and the pieces were mixed and then minced once in a mincer with a 3-mm mincer plate. The pH of the meat was 5·5–5·7. The minced meat was divided into 450-g unit packs, placed in moisture-proof plastic bags, closed under vacuum and stored at −25°C. When needed, the samples were thawed for 24 h at +4°C.

Preparation of batter

Thawed lean beef (450 g) was comminuted for 20 s in a 2-litre bowl cutter equipped with eight blades. Then 450 g of crushed ice and 22 g of curing salt (table salt containing 0·1 g of $NaNO_2$) were added and the mixture was comminuted for another 160 s. The temperature of the batter was monitored continuously with a thermometer (Pt82) which was placed permanently at the centre of the bowl, 3 mm over its surface. The maximum temperature was 6°C.

After the addition of 380 g of ground fat the comminution was continued for a further 180 s. The maximum temperature of the batter at this point was about 12°C.

At the end of the comminution process six preweighed cans (size 99/36) were filled with about 200 g of the fat-containing batter. All cans were closed and heated for 35 min in a boiling water-bath (core temperature about 90°C). After cooling in running tap-water the cans were stored at 0°C for 24 h. The batters contained about 10% ± 1·5% protein, 30% ± 3% fat and 1·7% ± 0·1% salt. All experiments were repeated three times.

Determination of jelly and fat separation

After warming the cans in a water-bath at 45°C for 1 h they were opened and the fluid in each can was collected in a 100-ml volumetric cylinder. The fluid jelly and fat, which separated well in the volumetric cylinder, were measured in millilitres and calculated as percentage of the original weight of batter. The mean value of the six cans was taken for each treatment.

Fat extraction

About 100 g of fat were taken from each sample just before its use in the batter. The fat was mixed with an equal quantity of purified seasand and about 20 g of anhydrous sodium sulphate. After intensive mixing, about 80 ml of diethylether were added and the mixture was stirred for about 10 min. The liquid phase was separated through a fine plastic sieve and was filtered through glass wool. The ether phase was removed by the use of a rotation evaporator. The separated fat was used immediately for determination of lipid oxidation.

Measurement of lipid oxidation

The 2-thiobarbituric acid (TBA) test and the peroxide value test were used to assess lipid oxidation. The TBA test was carried out in duplicate samples according to the method described by Schmidt (1959). Results are expressed as TBA number, i.e. milligrams of malondialdehyde per kilogram of fat. The peroxide value test was carried out in duplicate samples according to the method described by Wheeler (1932). Results are expressed as peroxide value, i.e. moles of O_2 per kilogram of fat.

pH measurement of fat

To avoid errors caused by changes in pH values of meat, fat and batter, the pH was measured. About 3 g of fat, batter or meat were homogenized with 10 ml of distilled water in a 25-ml flask using a homogenizer (E. Bühler, Tübingen). The homogenate was filtered through a filter paper, and the pH was measured in the filtrate with a digital pH-meter equipped with a combined glass electrode. The electrode remained in the filtrate until the pH value became constant.

Statistical analysis

Experiment A was carried out in three replications. Data for jelly and fat separation were analysed by Student's *t*-test. Simple linear regression was

used to determine the relationship between TBA number and peroxide value for all treatments.

RESULTS AND DISCUSSION

Effect of additives on fat oxidation and batter stability

Influence on fat oxidation

Without any additive the peroxide values and TBA numbers increased during storage. The TBA number doubled within 8 days, steadily increasing, and the peroxide values increased only after a storage period of 8 days (Figs 1 and 2).

The effect of nitrite, nitrate, DL-α-tocopherol and 6-*O*-palmitoyl-L-ascorbic acid on the oxidation of fat is also shown in Figs 1 and 2. Sodium nitrite has a pro-oxidant effect on the oxidation of the fat during storage. The peroxide value increased at the first day of storage but remained almost constant after the eighth day. The pro-oxidant effect of nitrite on the oxidation of fat is evident with the peroxide value; with TBA numbers the differences from control are small.

The oxidative effect of nitrite may occur because of the existence in the fatty tissue of small amounts of free iron ions and other ions of transition elements. In muscle tissue these ions are bound or chelated by charged groups of amino acid side-chains of the proteins and are not available as

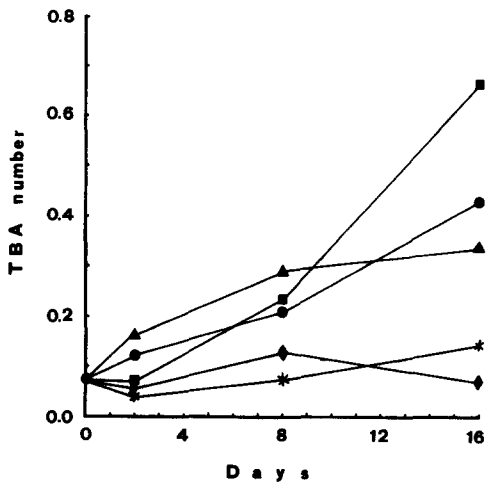

Fig. 1. Effect of additives on oxidation of pork back fat during storage. ●, Control; ▲, sodium nitrite; ■, sodium nitrate; ◆, 6-*O*-palmitoyl-L-ascorbic acid; ★, DL-α-tocopherol. (For concentrations see 'Materials and Methods'.)

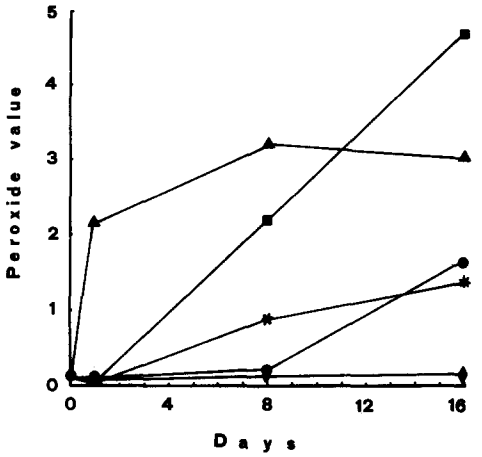

Fig. 2. Effect of additives on oxidation of pork back fat during storage. Symbols as in Fig. 1.

catalysts or intermediates for oxidation processes. In fatty tissue this sequestration does not take place. According to Morrissey & Tichivangana (1985), the antioxidative activity of nitrites in meat products is due to the formation of nitrosomyoglobin with meat myoglobin; this has antioxidant properties and on heating forms nitrosylhaemochrome, which blocks the catalytic activity of haem iron and prevents its release. Furthermore, Morrissey & Tichivangana, as well as MacDonald *et al.* (1980), postulated a 'chelation' of ferrous (Fe^{2+}) ions by nitrite.

The TBA number and the peroxide value of the fat treated with nitrate were unchanged at the first day of storage but then a nearly linear increase was observed up to the 16th day. This increase in peroxide number related to nitrate and nitrite may be the result of partial reduction of nitrate to nitrite by the action of micro-organisms, which may be present in the fat. Another possibility which seems likely is an as yet unknown chemical reaction in which nitrate is involved.

The oxidative effect of nitrite and nitrate, in the form of curing salt, on the oxidation of fatty tissue has been observed by Lea (1934) and Coxon *et al.* (1986). Ellis *et al.* (1968) have also shown that nitrite has a pro-oxidant effect on bacon.

Palmitoyl-L-ascorbic acid as an antioxidant, in a concentration of 1500 ppm, showed the lowest TBA number and peroxide value and exhibited only a small increase in TBA number with time of storage.

DL-α-Tocopherol, which is also a fat-soluble antioxidant, although it showed TBA numbers as low as the palmitoyl-L-ascorbic acid, had a peroxide value more or less equal to that of the control treatment. According to Henkel (1986), cited by Dziezak (1986), α-tocopherol has lower stabilizing activity for fat than the other forms of tocopherol.

Zipser & Watts (1962) and Shahidi (1989) reported that TBA numbers are not valid in cured meat products as nitrite inhibits the reaction between 2-thiobarbituric acid and malonaldehyde. This may account for the smaller differences in TBA number with time of storage and additive in Fig. 1 compared with peroxide value (Fig. 2); in the latter case, the addition of nitrite and nitrate led to considerable increases.

Nevertheless, we found a correlation coefficient of $r = 0{\cdot}58$ (significant at the 1% level) between the TBA number and the peroxide value over all treatments.

Influence on batter stability

In the experiments a batter composition with a rather limited protein and salt content was deliberately chosen, to enhance the effects of additives. In particular, improvements in water and fat binding could be observed clearly. Therefore the unusually high jelly release was intended.

Without any additive, as shown in Fig. 3, the changes of jelly release with time of storage were rather small (minimum 28%, maximum 29·7%). Fat rendering, however (Fig. 4), increased steadily from 1·2% at day 0 (slaughter-fresh) to 5% after 16 days of storage. This is in agreement with the observations of Bloukas & Honikel (submitted).

The effect of the additives used on jelly and fat separation of batters is shown in Figs 3–5. In contrast to the increase in fat oxidation, the addition of sodium nitrite led to a remarkable decrease in jelly and fat separation of batters, which was very pronounced after the second day of storage compared with control and could be repeated in all three experiments.

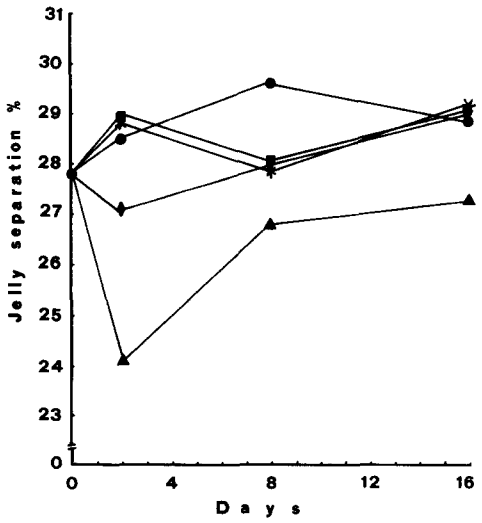

Fig. 3. Effect of additives on jelly separation of batters. Symbols as in Fig. 1.

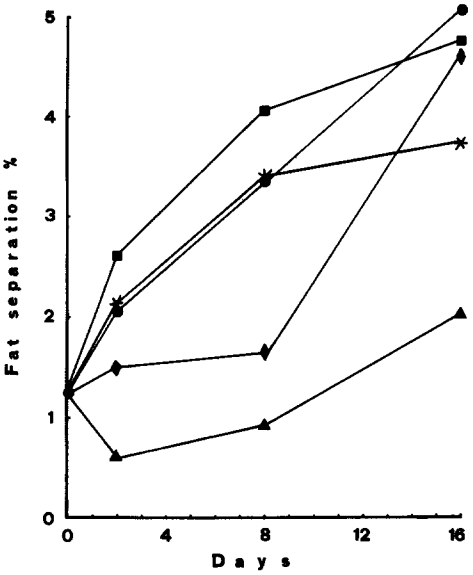

Fig. 4. Effect of additives on fat separation of batters. Symbols as in Fig. 1.

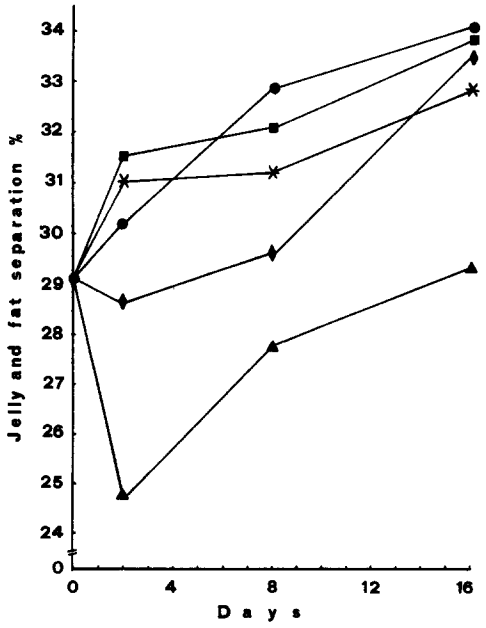

Fig. 5. Effect of additives on jelly and fat separation of batters. Symbols as in Fig. 1.

The addition of palmitoyl-L-ascorbic acid also gave a small improvement in jelly and fat separation compared with control up to the eighth day. The differences were not consistent in all three experiments.

The addition of nitrate and DL-α-tocopherol to the fat showed no clear effect on jelly and fat separation compared with control.

For all treatments, storage of the fat had no significant effect on jelly separation ($P > 0{\cdot}05$). The only exception recorded was between day 0 and day 16 of storage, where the effect was significant ($P < 0{\cdot}05$). In contrast to jelly separation, the storage time of fat was found to exert a significant effect ($P < 0{\cdot}05$) on fat separation of batter.

Figure 5 shows the influence of the additives on total cookout (jelly and fat separation). Over the whole period of fat storage in all three experiments the addition of nitrite reduced the cookout; on day 2 an 18% reduction in mean values compared with control was observed, and on day 16 a 12% reduction. The addition of palmitoyl-L-ascorbic acid gave a smaller decrease in total cookout, except on day 16. DL-α-Tocopherol and nitrate had a rather small cookout-reducing effect.

Effect of nitrite concentration

The authors are aware that the addition of 2000 ppm nitrite to fat is beyond any legal limits. Without breakdown of nitrite during storage there would be 400 ppm nitrite in the batter before cooking. This is about 2–4 times as much as is allowed by the regulations of various countries. An addition of 500–800 ppm of nitrite to the back fat in the batter composition used would

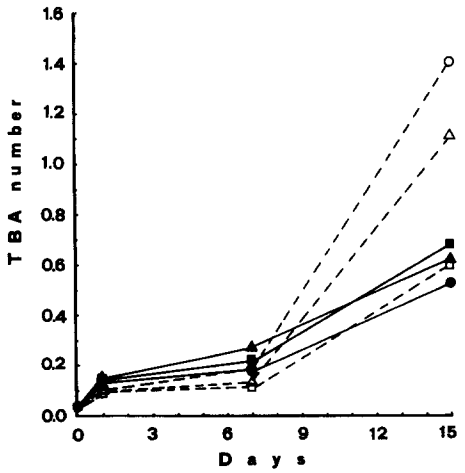

Fig. 6. Effect of nitrite level on oxidation of pork back fat during storage. ●, Control; ▲, 62·5 ppm; ■, 125 ppm; ○, 375 ppm; △, 1125 ppm; □, 2250 ppm.

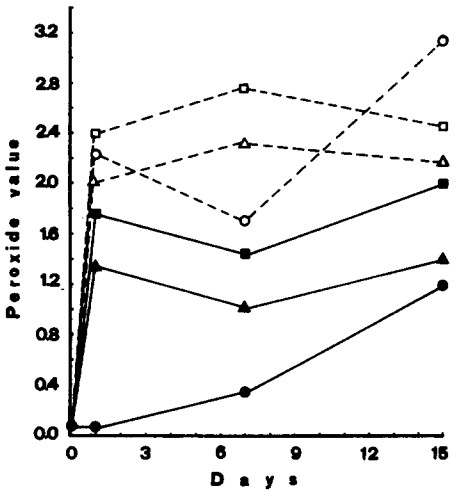

Fig. 7. Effect of nitrite level on oxidation of pork back fat during storage. Symbols as in Fig. 6.

lead to acceptable limits of 90–150 ppm of nitrite in the final batter. To prove the effect of lower concentrations of nitrite we carried out experiment B.

The effect of nitrite concentration on the oxidation of pork back fat is given in Figs 6 and 7. The addition of nitrite to the fat has a remarkable pro-oxidant effect which is indicated clearly by the peroxide value. It is also remarkable that from the first to the seventh or even 15th day after addition the peroxide value remained more or less constant. The TBA numbers, however, increased after 7 days of storage and the effect was smaller than that on peroxide values.

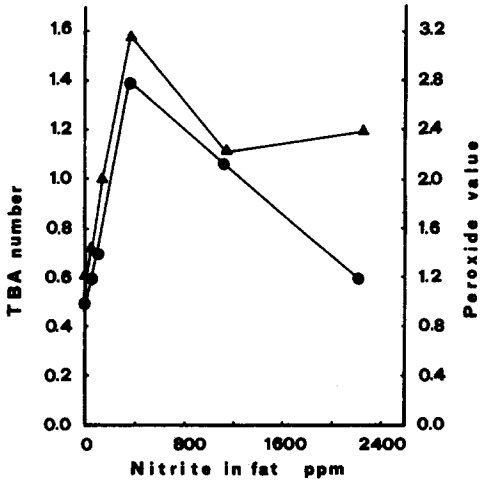

Fig. 8. Effect of nitrite level on oxidation of pork back fat at the 15th day of storage. ●, TBA number; ▲, peroxide value.

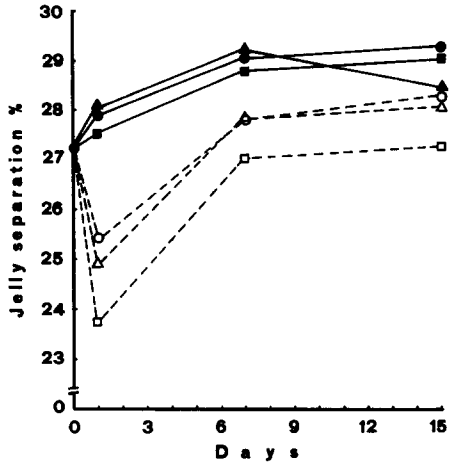

Fig. 9. Effect of nitrite level on jelly separation of batters. Symbols as in Fig. 6.

Addition of nitrite up to 375 ppm increased peroxide values and TBA numbers, as shown in Fig. 8 for day 15. However, higher additions of nitrite led again to reduced peroxide values and TBA numbers, but were higher at 2250 ppm nitrite than without addition. Obviously, concentration-dependent mechanisms are involved. Another mode of action was observed on jelly and fat release. This confirms partially the findings of Lea (1934), who showed that the pro-oxidant effect of nitrite on the oxidation of fatty tissue increases with increase in its concentration.

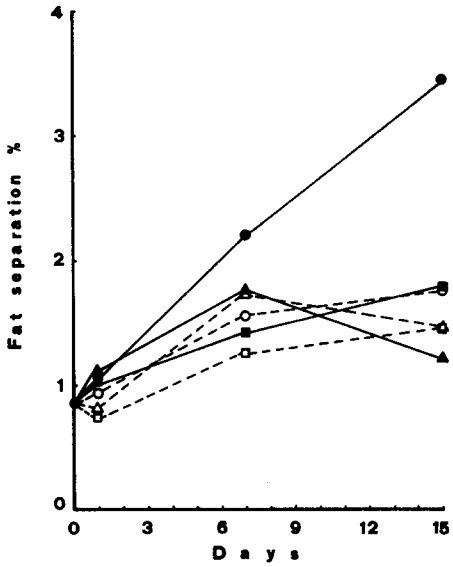

Fig. 10. Effect of nitrite level on fat separation of batters. Symbols as in Fig. 6.

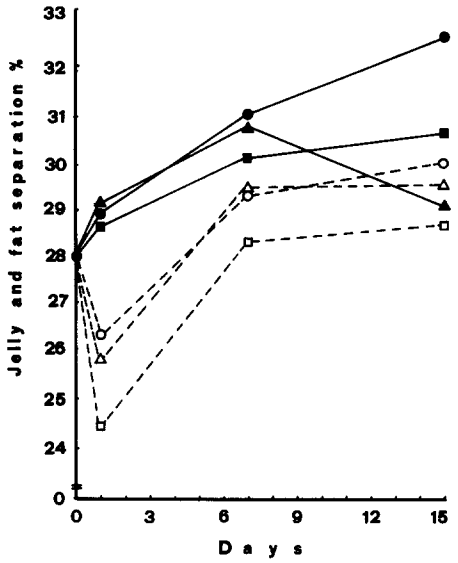

Fig. 11. Effect of nitrite level on jelly and fat separation of batters. Symbols as in Fig. 6.

The effect of nitrite concentration on jelly and fat separation in batters is shown in Figs 9–11. Concentrations of nitrite in the fatty tissue up to 125 ppm had little influence on jelly release, but concentrations higher than 125 ppm showed a remarkable decrease in jelly separation on the first day (Fig. 9). The higher the nitrite concentration, the lower the jelly separation. After the first day the jelly separation increased again up to the eighth day, but remained lower than in the control treatment.

The effect of any nitrite addition becomes more obvious on fat separation (Fig. 10). All nitrite treatments showed lower fat separation than the control treatment, especially on the eighth and 15th days, but there were no clear differences for the various concentrations of nitrite in the fat.

CONCLUSIONS

From the results in both sets of experiments, it is clear that fat oxidation has little effect on jelly and fat separation of batters. Antioxidative agents which prevent rancidity (6-*O*-palmitoyl-L-ascorbic acid and DL-α-tocopherol) have no effect either on the batter stability. Nitrite, which has antioxidative effects in meat products, promotes pure back fat rancidity but reduces jelly and fat separation of batters. The relationship, however, is rather poor (Figs 1 and 2 vs Fig. 5, or Figs 6 and 7 vs Fig. 11). A hypothesis on the mode of action of nitrite and nitrate on fat oxidation and the stabilizing action of nitrite on

batter cookout would be pure speculation at this time. Further experiments are needed.

However, from these experiments, and those reported by Bloukas & Honikel (in press), it becomes evident that the excellent binding properties of slaughter-fresh fat compared with fat stored for 1–16 days are not due to the progress in fat oxidation.

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