ANTIOXIDATIVE ACTIVITY OF SPICES IN INTERMEDIATE MOISTURE MEATS

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ABSTRACT: Modern intermediate moisture (IM) meats are a potential means of preservation where storage and distribution facilities are not available. Lipid oxidation, or development of rancidity, is one of the major problems found in such products. On the other hand, it is known that certain spices possess antioxidative activity. Thus, this study was designed to evaluate chillie powder, cumin, cloves and black pepper as antioxidants in IM pork meat. Samples were prepared from pork Longissimus dorsi muscle; trimmed of all visible fat and ground. IM water activities (a_w) were obtained by the addition of NaCl, glucose or sucrose. Samples with added spice (1%) were loosely wrapped with polyethylene and stored for four days at 4 ^{o}C. Water activity, pH and rancidity were measured after storage. Rancidity was expressed as mg of malonaldehyde per Kg of sample by means of the thiobarbituric acid (TBA) test. Results showed that cloves were the most effective antioxidants followed by chilli powder and black pepper. Cumin showed little effectiveness as antioxidant. Sodium chloride, sucrose and glycerol were effective in lowering a_w, though at high concentrations (15%, 62%, 36%, respectively). NaCl enhanced the development of rancidity.

SUMMARY: Black pepper, chilli powder, cloves and cumin were tested as antioxidants in both fresh and intermediate moisture (IM) ground pork. Low water activities (a_w=0.85) were attained employing NaCl, glycerol and sucrose as humectants. It was found that cloves had the most effective antioxidative effect followed by chilli powder and black pepper in both meat systems. Cumin had little antioxidative activity. NaCl was found to be more effective in lowering a_w than glycerol and sucrose, however, to achieve the desired a_w it markedly enhanced rancidity (expressed as TBA numbers). All humectants were required in large amounts.

INTRODUCTION: Interest in meat preservation for developing countries has increased in recent years. The small number of animals, the lack of suitable handling as well as storage and distribution facilities prevent meat and meat products reaching the table of many families. Furthermore, the technology and equipment needed are expensive, which diminishes the availability of such food products even more. Intermediate moisture (IM) meats offer a potential solution and have been studied over the last 30 years.

Some progress has been made towards both understanding and developing IM meat, however, more knowledge is needed to manufacture fully stable IM meat products. On the other hand, consumers in general, seek healthier food; this involves the use of naturally-occurring substances in meat products and not synthetic or non-nutritive ones. It was, thus, of interest to know whether the naturally-occurring compounds in spices are able to inhibit or retard the development of rancidity in IM pork, where due to long storage periods, lipid oxidation is a problem likely to be present.

The study was divided into three stages. In the first, effectiveness of spices on fresh minced pork was tested; in the second, the required amount of different humectants was stablished in order to obtain a water activity (a_w) of 0.85 which corresponds to the maximum limit accepted for IM products (Potter, 1970; Heidelbauch *et al.*, 1975; Robson, 1976). In the final stage, a low a_w product containing spice was prepared to estimate the antioxidative activity of spices in intermediate moisture pork.

MATERIALS AND METHODS : Post rigor *Longissimus dorsi* from pork carcasses was obtained from the University's slaughterhouse. The meat was trimmed of all visible fat and connective tissue and ground in a 3-MK-4 Kenwood mixer (4 mm diameter plate). This procedure was followed in all three stages of the experiment.

Stage 1. Testing Spices as Antioxidants. Ground black pepper and chilli powder were added (1%) to different 100g batches of meat, leaving one batch blank as a control. Each lot was manually mixed until homogeneous distribution of the

spice was achieved. They were then divided into 4 samples, spread evenly in a sample tray to a depth of 1.5 cm and loosely wrapped with oxygen permeable polyethylene. All samples were stored for 4 days at 4°C (Witte et al., 1970; Rhee et al., 1983).
Ground cumin and ground cloves were tested following the same procedure. Lipid oxidation and pH were measured before adding spices and after the storage period.

Stage 2. Preparation of Intermediate Moisture Pork. Batches of trimmed minced pork were mixed with either sodium chloride or glycerol or sucrose (all analytical grade) in the proportions shown in Table 1 to produce four 100g lots. Each one was divided into two (to duplicate results), loosely wrapped and stored for 24 hours (Obanu et al., 1975, Obanu et al., 1975a) at 4°C. Water activity and lipid oxidation were measured before adding the humectants and at the end of the storage period.

TABLE 1. Concentration of humectants tested to achieve an a_w=0.85 in minced pork.

NaCl (%)	Sucrose (%)	Glycerol (%)
0	0	0
5	10	10
10	15	15
15	20	20
20	25	25
25	30	30
30	35	35
--	40	40
--	45	45
--	50	50
--	55	55
--	60	60
--	65	--
--	70	--

Stage 3. Intermediate Moisture Pork Containing Spices as Antioxidants. Black pepper, cloves or chilli powder (1%) were added to meat at an a_w=0.85, generating nine samples (3 spices x 3 humectants) and a control. All ten samples were loosely wrapped and stored at 4°C for 4 days. Lipid oxidation, water activity and pH were measured in quadruplicate at the end of the storage period.

pH was measured using a glass electrode and buffers at pH 4 and pH 7.

Lipid oxidation was estimated using the 2-thiobarbituric acid (TBA) test (Tarladgis et al., 1960) modified by Hoyland and Taylor (1989). The TBA value was expressed as mg of malonaldehyde per Kg of sample using the conversion factor (Tarladgis et al., 1960) for this particular system (K=12.7):

$$\text{TBA value (mg malonaldehyde/Kg sample)} = O.D._{sample} \times 12.7$$

Water activity measurements were made with a Decagon CX-1 Water Activity System tempered at 25°C.

Statistical Analysis was carried out in all data using a DataLab Manager (EMF Software, 1984) computer program. The confidence intervals used were $p<0.05$ and $p<0.01$.

RESULTS AND DISCUSSION: Stage 1. Table 2 shows the change in pH in the first stage. Differences were significant between initial (day 0) and final (day 4) pH in the controls and samples containing cumin and cloves ($p<0.01$) and in samples with chilli powder ($p<0.05$). However, no significant differences were found on storage in samples with black pepper, pH remaining constant. The decrease in pH was greater in samples containing cumin ($p<0.01$) than that observed in the control.
Although the pH of the meat containing cloves decreased, the decrease was not significantly different ($p<0.05$). That was also the case of samples containing chilli powder.
It was thought that such decrements could have been due to the addition of spices. Spice solutions (1%) were then made to determine their pH. The values were: 4.64 for chilli powder; 5.74 for cumin; 6.20 for black pepper, and 3.92 for cloves. This explains a) the decrease of pH in samples with chilli powder, cumin and cloves since these were more acidic than the original plain meat, and b) the maintenance of pH in samples with black pepper as it was less acidic than meat. However, both controls also showed lower pH, which, although no microbiological analyses were performed, could be explained in terms of the microbial flora present in the meat where no inhibitory effect from spices (Savik and Bern, 1983; Gerhardt, 1984; Korkzak et al., 1988) was present.

Concerning lipid oxidation (Table 3), significant differences in TBA numbers were expected between the controls and samples containing spice. This was observed in samples with cloves ($p<0.05$), with black pepper ($p<0.01$) and with chilli powder ($p<0.01$).
In the other hand, rancidity was expected to increase with time in all samples, though to different extents due to the effect of the particular spice present.
Suprisingly, no statistical difference ($p<0.05$) was found between the initial and final TBA numbers of samples with cloves, what suggests no development of rancidity.
Chilli powder and black pepper exhibited interesting results as TBA numbers in samples containing them decreased after storage. This leads one to believe that the concentrations of certain carbonyl compounds decreased. Obanu and co-

workers (1975b) suggested that such carbonyls could take part in further reactions so that they would not be free to react with TBA.

Cumin was found to have little antioxidative effect. Similar results were obtained by Chipault *et al.*, (1952, 1955) in lard.

TABLE 2*. Initial and final pH's[1] of minced, trimmed *L. dorsi* from pork with and without spices (1%).

	Control[2]	Chilli[3] powder	Black pepper	Control[2]	Cumin[2]	Cloves[2]
Day 0	5.58	5.58	5.58	6.20	6.20	6.20
Day 4	5.43	5.48	5.58[5]	5.86	5.72[4]	5.84

TABLE 3*. Initial and final TBA numbers[1] of minced, trimmed *L. dorsi* from pork with and without added spices (1%).

	Control[2]	Chilli[2] powder	Black[3] pepper	Control[2]	Cumin[3]	Cloves
Day 0	1.24	1.24	1.24	0.17	0.17	0.17
Day 4	5.94	0.49[4]	0.78[4]	0.56	0.33	0.20[5]

* Key:
1) Mean of four replicates
2) Significant difference ($p<0.01$) between initial and final pH's
3) Significant difference ($p<0.05$) between initial and final pH's
4) Significantly different ($p<0.01$) to control after storage
5) Significantly different ($p<0.05$) to control after storage

Stage 2. Figure 1 shows the isotherms constructed with data collected in order to set the amount of humectant required to attain an a_w=0.85 in fresh ground pork meat. It can be seen clearly that NaCl was the most effective humectant, requiring 15% for a_w 0.85 against glycerol (36%) and sucrose (62%). However, TBA numbers were far higher in meat containing NaCl than with the other humectants (Figure 2).

That NaCl enhanced rancidity is supported by the findings of many authors (Chang et al., 1950; Watts, 1962; Love et al., 1971; Greene et al., 1975; Schwartz et al., 1976; Neer et al., 1977; Huffman et al., 1981; Rhee et al., 1983; Keeton, 1983). It is known that iron and haem pigments are active pro-oxidants (Pearson et al., 1977; Verma et al., 1985) and the possibility that iron impurities might have been present in the NaCl was investigated. It was found that NaCl had more iron (<4 ppm) than both glycerol (<0.05 ppm) and sucrose (<1 ppm). Moskovits et al. (1960) pointed out that 10 ppm of iron can accelerate rancidity. Whether the accelerated rancidity was due to the iron content of the NaCl or whether NaCl causes changes in the meat which accelerate oxidation is not clear.

TABLE 4*. pH[1] determined in minced, trimmed pork (a_w=0.85) with and without added spice (1%) after 4 days storage (4°C).

	Control	Cloves	Black pepper	Chilli powder	Mean[2]
NaCl	5.37	5.20	5.30	5.22	5.24[3]
Sucrose	5.37	5.31	5.38	5.28	5.33
Glycerol	5.37	5.18	5.33	5.23	5.25[4]
Mean	5.37	5.23[3]	5.34	5.27[4]	

TABLE 5*. TBA numbers[1] determined in minced, trimmed pork (a_w=0.85) with and without added spice (1%) after storage (4°C).

	Control	Cloves	Black pepper	Chilli powder	Mean[2]
NaCl	1.008	0.356	2.046	0.903	1.101
Sucrose	1.008	0.187	0.188	0.168	0.181[4]
Glycerol	1.008	0.178	0.321	0.153	0.217[4]
Mean	1.008	0.240[4]	0.851	0.408	

*Key
1) Mean of 4 replicates
2) Without control
3) Significantly different ($p<0.05$) to control
4) Significantly different ($p<0.01$) to control

FIGURE 1. Effectiveness of humectants in pork after 24 hours storage (4°C)

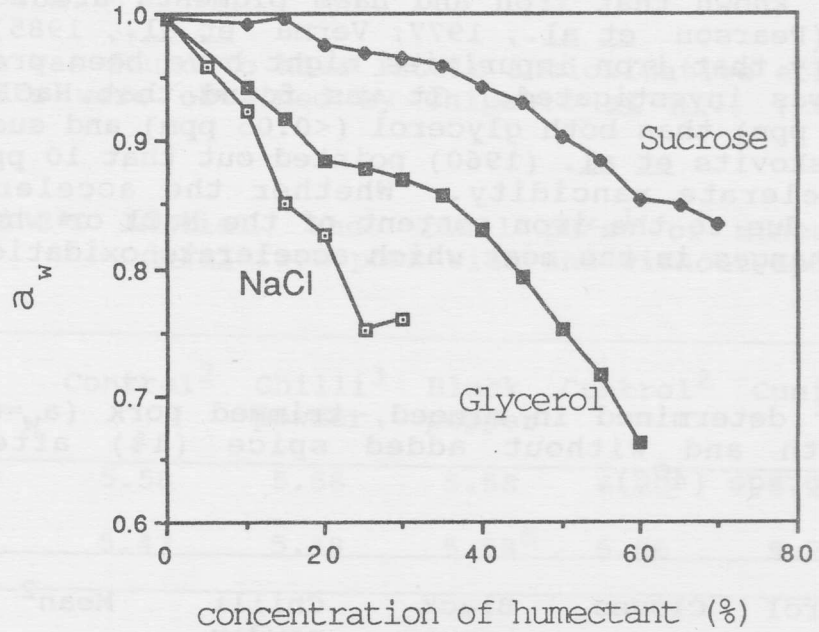
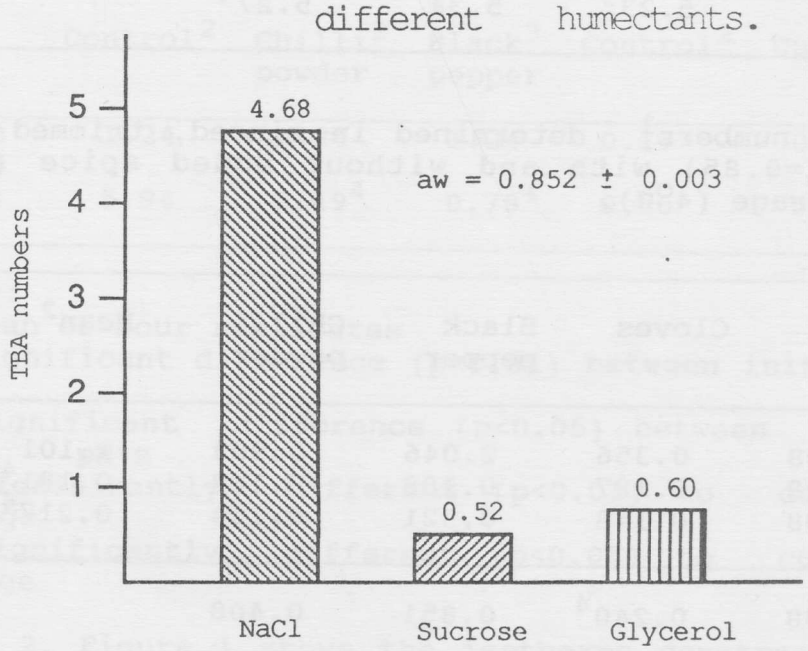


FIGURE 2. TBA numbers of pork with aw = 0.85 attained with different humectants.



Stage 3. The initial pH of the meat was 5.61, and it is clear from Table 4 that pH decreased in all samples during storage with significant differences between them. Again, decrease in pH is an advantage in meat systems as it helps to avoid microbioloical growth. Unfortunately, such decrements cannot be explained in these terms also because of the low availability of water (a_w=0.85) in the samples. Furthermore, it has been found that spices may inhibit the growth of certain microorganisms as was mentioned previously.
Table 5 shows the TBA numbers determined in minced, trimmed pork (a_w=0.85) with and without added spice (1%) after 4 days storage. Only samples with cloves, regardless the humectant employed, were significantly different ($p<0.01$) to the other samples, i.e., cloves retarded development of rancidity. Moreover, Herrmann (1981) reported that cloves showed enhanced antioxidative activity in the dark and all samples were stored in dark.
Nakatani (1988) isolated certain substances from spices, all containing several hydroxyl groups attached to an aromatic ring. It was suggested (Gerhardt, 1984) that such groups play important roles as antioxidants. These or similar substances may also be present in cloves, chilli powder and black pepper.

Comparing analysis between humectants but within spices, it was observed that, broadly speaking, samples in which a_w was controlled by NaCl, developed rancidity to practically the same extent of the control ($p<0.05$). On the contrary, samples containing either glycerol or sucrose did not show increased lipid oxidation ($p<0.01$).

In summary, spices were found to be effective antioxidants both in fresh and IM pork, cloves being the most efficient followed by chilli powder and black pepper. Cumin was found to have little antioxidative activity. Hence, it is possible that spice extracts could be used both as antioxidants and as part of the seasoning mixture in the manufacture of IM meat products.
Despite the fact that NaCl, glycerol and sucrose depleted a_w, the concentrations required were high, therefore, it is suggested that blends of humectants might be investigated to find a more suitable mix, which could lower the a_w efficiently and/or without imparting any undesirable flavour to the product, taking advantage of salt's efficiency and glycerol's capability to inhibit certain microorganisms (Plitman et al., 1973).

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