F
N
P

Journal of FOOD PROCESSING and PRESERVATION

Edited by
T.P. LABUZA

FOOD & NUTRITION PRESS, INC.
WESTPORT, CONNECTICUT 06880
USA

VOLUME 5, NUMBER 4 DECEMBER 1981

JOURNAL OF FOOD PROCESSING AND PRESERVATION

All articles for publication and inquiries regarding publication should be sent to Prof. T. P. Labuza, University of Minnesota, Department of Food Science and Nutrition, St. Paul, MN 55108 USA.

All subscriptions and inquiries regarding subscriptions should be sent to Food & Nutrition Press, Inc., 265 Post Road West, Westport, Connecticut USA.

One volume of four issues will be published annually. The price for Volume 5 is $55.00 which includes postage to U.S., Canada, and Mexico. Subscriptions to other countries are $65.00 per year via surface mail, and $72.00 per year via airmail.

Subscriptions for individuals for their own personal use are $35.00 for Volume 5 which includes postage to U.S., Canada, and Mexico. Personal subscriptions to other countries are $45.00 per year via surface mail, and $52.00 per year via airmail. Subscriptions for individuals should be sent direct to the publisher and marked for personal use.

The *Journal of Food Processing and Preservation* is listed in *Current Contents/Agriculture, Biology & Environmental Sciences (CC/AB).*

The *Journal of Food Processing and Preservation* (USPS 456-470) is published quarterly by Food & Nutrition Press Inc. — Office of publication is 265 Post Road West, Westport, Connecticut 06880 USA.

Second class postage paid at Westport, CT 06880.

POSTMASTER: Send address changes to Food & Nutrition Press, 265 Post Road West, Westport, CT 06880.

JOURNAL OF FOOD PROCESSING AND PRESERVATION

Journal of
FOOD PROCESSING
and
PRESERVATION

VOLUME 5
NUMBER 4

Editor: T. P. LABUZA

FOOD & NUTRITION PRESS, INC.
WESTPORT, CONNECTICUT 06880 USA

Food & Nutrition Press, Inc.
Westport, Connecticut USA

ISSN: 0145-8892

Printed in the United States of America

CONTENTS

EFFECT OF METAL IONS ON RESIDUAL NITRITE[1]

M. LEE, R. G. CASSENS and O. R. FENNEMA

College of Agricultural and Life Science
University of Wisconsin
Madison, WI 53706

Received for Publication May 10, 1981

ABSTRACT

The effect of various metal ions on residual nitrite was studied in both a meat system and a model system. Reducing ability, nitrosylhemochrome, nitrate and pH were determined in an attempt to explain the mechanism of loss of nitrite in the system. The control meat system contained 2% NaCl and 156 ppm of $NaNO_2$ while treatment groups had 0.05% of metal ions (Fe^{++}, Fe^{+++}, Cu^{++}, Zn^{++}, Ca^{++}, and Mg^{++} as chloride salts) substituted for the same amount of NaCl. The results showed that ferrous (Fe^{++}), ferric (Fe^{+++}), cupric (Cu^{++}) and Zinc (Zn^{++}) ions caused a depletion of nitrite while calcium (Ca^{++}) and magnesium (Mg^{++}) ions did not have any effect on residual nitrite. The model system consisted of 25 mM PIPES buffer at pH 5.8 to which was added 156 ppm $NaNO_2$ with or without 0.05% metal ions. Only ferrous (Fe^{++}) ion decomposed more nitrite than the control after heating. Considering the results from both systems, the mode of action for each ion appeared to be different; ferrous ion appeared to act directly as an electron donor, ferric ion acted like ferrous after being reduced, cupic ion appeared to act as an electron donor after being reduced and then as an electron acceptor, and zinc ion seemed to be effective only by decreasing the pH of the system.

INTRODUCTION

Sodium chloride is an integral component of meat processing, and its addition to meat is known to increase free bivalent metal ions (Swift and Berman 1959; Ando *et al.* 1973). Metal ions are known to be a factor in water-holding capacity of meat (Swift and Berman 1959; Ando *et al.* 1973). Gibson (1943) showed that metal ions (Cu^{++} and Fe^{++}) exerted a catalytic effect on the rate of reduction of methemoglobin in the presence of ascorbic acid. Since then, several studies have revealed the enhancing effect of metal ions such as Fe^{++}, Fe^{+++}, Cu^{++} and Zn^{++} in the presence of ascorbic acid (Weiss *et al.* 1953), and Fe^{++} in the presence of cysteine or ascorbic acid (Sielder and Schweigert 1959; Reith and Szakaly 1967) on the formation of the cured color

[1] Research supported by the College of Agricultural and Life Science by the Food Research Institute and by the American Meat Institute. Muscle Biology Laboratory manuscript number 149.

pigment. Seman *et al.* (1980) showed that partial replacement of NaCl by $MgCl_2$ or KCl in bologna preparation was possible without impairing the functionality of NaCl. Tompkin *et al.* (1978) reported that among metal ions examined (Fe^{++}, Fe^{+++}, Mg^{++}, Mn^{++} and Zn^{++}), Fe^{++} and Fe^{+++} caused a loss of botulinal inhibition by nitrite in processed meat. It has been reported that the level of residual nitrite is likely to be a factor in botulinal inhibition in cured meats (Christiansen *et al.* 1978; Tompkin 1978). However, the level of residual nitrite is known to be associated with formation of nitrosamines which are carcinogens. Therefore, the control of residual nitrite has assumed considerable importance.

Many factors affect the level of residual nitrite in cured meat. These factors include pH (Nordin 1969), temperature of cooking and storage (Olsman and Krol 1972), meat content (Olsman and Krol 1972), properties of meat (Lee and Cassens 1976), reducing substances (Fox and Nicholas 1974) and metal ions (Olsman and Krol 1972; Olsman 1974; Ando 1974). Since meat has an endogenous reducing ability (Walters and Taylor 1963; Rose and Peterson 1953; Ando and Nagata 1970), and it has been suggested that oxidation of nitrite to nitrate occurred only at the higher redox potential before heating meat (Mhler 1971), many researchers postulated that nitrite disappeared through a reductive process mediated by electron donors such as sulfhydryl groups and trace metal ions (Rose and Peterson 1953; Olsman and Krol 1972; Olsman 1974).

An effect of metal ions on depletion of nitrite has been noticed in various experiments involving sulfhydryl group alkylation and EDTA (Olsman 1974), ferrous ion and EDTA (Olsman and Krol 1972) and metal ions (Ca^{++}, Mg^{++}, Zn^{++}, Fe^{++}, and Fe^{+++}) (Ando 1974).

In our experiments, the effects of various metal ions (Fe^{++}, Fe^{+++}, Cu^{++}, Ca^{++}, Mg^{++}, and Zn^{++}) on residual nitrite were determined, and an effort was made to explain the mechanism of loss of nitrite by study of conditions in the system.

MATERIALS AND METHODS

Posterior limbs were collected from pigs slaughtered at the University of Wisconsin Meat Laboratory, stored frozen at -23°C and thawed at 2-4°C prior to use. All of the muscles of the limb were ground through a plate with holes of 0.4 cm diameter, mixed with salt and metal ions (or without metal ions) for 3 min and then with $NaNO_2$ for an additional 2 min. The control contained 2% NaCl and 156 ppm of $NaNO_2$ while treatment groups had 0.05% metal ion salts replacing the same portion of NaCl. Metal ion sources were $FeCl_2 \cdot XH_2O$ (di and tetrahydrate), $CaCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$ and $ZnCl_2$ (Mallinckrodt, Inc.), and $FeCl_3$ and $CuCl_2 \cdot 2H_2O$ (Matheson Coleman and Bell Manufacturing Chemists). The mixtures were packed into screw-

capped glass jars and cooked at 80°C in a water bath until an internal temperature of 65°C was reached. After cooking, the samples were cooled for 24 h at 2-4°C and then mixed well. They were stored in screw-capped glass jars at 2-4°C for a one-month period during which time they were sampled periodically.

Nitrite analysis was as described by Lee and Cassens (1980). Nitrate was measured by measuring nitrite before and after reduction with spongy cadmium and expressing the difference as nitrate. Five ml of buffer at pH 9.7 (Follett and Ratcliff 1963) and 0.5 gram of spongy cadmium were added to 20 ml of the filtrate. The mixture was shaken mechanically for 3 min to reduce nitrate to nitrite. The spongy cadmium was prepared by putting zinc rods into 20% (W/V) cadmium sulfate solution. Spongy cadmium on the zinc rods was removed, transferred to 0.2N HCl and then blended for 1-2 s so that the bulk of it was reduced to 8-40 mesh size which was used for nitrate reduction. The spongy cadmium (mesh size 8-40) was washed with 0.2N HCl for 30 min in order to regenerate it and then with distilled water before use. After use, it was washed with 0.2N HCl and then stored in distilled water.

Reducing ability was measured by the method of Ando and Nagata (1970) with the following modification. Two grams of sample was homogenized with 10 ml of 25 mM PIPES [piperazine-n, n-bis (2-ethanesulfonic acid)] buffer at pH 5.8. Five ml of homogenate was transferred to a 10 ml-volumetric flask and then 2 ml of 5 mM $K_3Fe(CN)_6$ was added. The mixture was incubated with occasional stirring for 1 hour at 2-4°C. After incubation, 0.1 ml of 0.5% ammonium sulfamate and 0.2 ml of 0.5M lead acetate were added to the mixture, and then the mixture was allowed to stand for 5 min at room temperature. After 5 min, 2.5 ml of 20% TCA was added and brought up to volume with distilled water. After another 5 min, the mixture was filtered through Whatman filter paper No. 42. The absorbance of the filtrate was measured at 420 nm after 30 min from the end of the incubation at 2-4°C. Reducing ability was expressed as absorbance of 1 mM of $K_3Fe(CN)_6$ at 420 nm minus absorbance of the sample.

Nitrosylhemochrome was determined according to Hornsey (1956). Ten grams of the sample were homogenized with 40 ml of acetone and 3 ml of distilled water in a screw-capped plastic bottle. The mixture was kept for 60 min with occasional stirring and then filtered through No. 42 Whatman filter paper. All preparations were done in a dark room. The absorbance of the filtrate was measured at 540 nm and this value was used directly to compare the nitrosylhemochrome content of various samples.

In the experiments with model systems, 156 ppm $NaNO_2$ and 0.05% metal salts were mixed with 25 mM PIPES buffer or with the homogenate of PIPES buffer 3:meat 2 (v/w) at pH 5.8, and heated at 80°C until an internal temperature of 70°C was reached. Nitrite was analyzed as described previously. For reducing ability, 2 ml of 5 mM $K_3Fe(CN)_6$ and 1 ml of the sample

solution were mixed in a 10 ml-volumetric flask and the measurement was carried out as described previously.

Statistical analysis were done by LSD according to Snedecor and Cochran (1973).

RESULTS

In the first experiment, ferrous (Fe^{++}), ferric (Fe^{+++}) and cupric (Cu^{++}) ions were studied, and the results for residual nitrite are shown in Fig. 1. Residual nitrite measured after mixing and heating was significantly lower ($P < 0.05$) in the ferrous, ferric or cupric ion treatment groups than in the control.

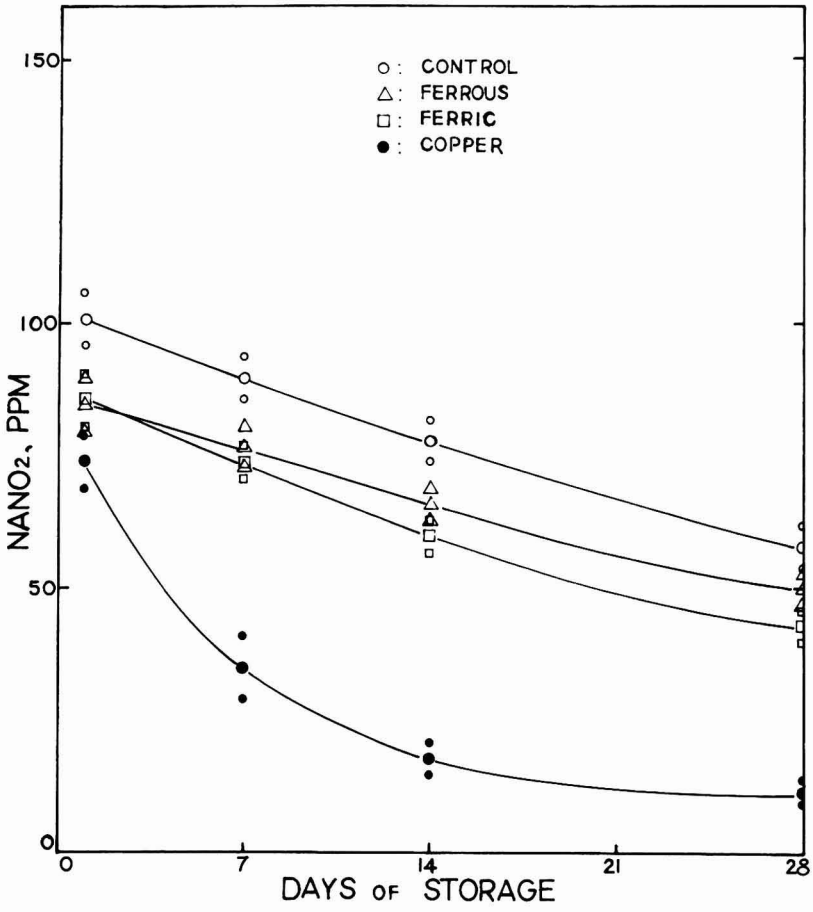

FIG. 1. EFFECT OF METAL IONS ON RESIDUAL NITRITE IN A MEAT SYSTEM. The first measurement was made at one day after mixing and heating. Standard deviations are shown with the appropriate symbols above and below the mean.

Among treatment groups, the cupric ion group had lower residual nitrite while there was no significant difference between ferrous and ferric ion groups. To see the rate of depletion during storage, slopes were calculated assuming that loss of nitrite during storage is a first order reaction (Olsman 1974; Nordin 1969). Values of slopes are -0.0087 for the control, -0.0089 for the ferrous ion treatment and -0.0112 for the ferric ion treatment. Statistical analysis showed that they were not significantly different. However, the cupric ion treatment group did not show a first order reaction. Table 1 shows that ferrous, ferric and cupric ion treatment groups had significantly lower pH values than the control after both mixing and cooking ($P < 0.05$).

Table 1. Effect of Treatment Method and Metal Ion on Change in pH of Meat Systems[1]

Treatment	Control	Ferrous	Ferric	Cupric
	pH Value[2]			
After mixing	5.57 ± 0.03^{A}	5.51 ± 0.03^{B}	5.43 ± 0.03^{C}	5.49 ± 0.03^{D}
After cooking	5.85 ± 0.02^{A}	5.78 ± 0.03^{B}	5.70 ± 0.04^{C}	5.73 ± 0.03^{C}

[1]Control had 2% NaCl while the others had 1.95% NaCl and 0.05% of metal ions as chloride salts.
[2]Mean of 3 observations ± SD. Values with different superscript letters in the same row are significantly different ($P < 0.05$)

However, results from a model system containing meat at the same pH showed that treatment groups still had lower residual nitrite level after cooking (Table 5).

In order to attempt to explain how metal ions influenced residual nitrite, the properties of reducing ability, the amount of nitrosylhemochrome and the level of nitrate present in the system were studied. As shown in Fig. 2. the cupric ion treatment group had the greatest reducing ability, the ferrous ion treatment group exhibited substantially less reducing ability and the control showed the least reducing ability at one day after cooking. To determine if the effects of metal ions on reducing ability in a meat system were caused directly by the metal ions, a nonbiological system, which contained a buffer incable of complexing metal ions, was studied. Only cupric ion showed substantial reducing ability in this model system.

The nitrosylhemochrome contents of the treatment groups are compared in Fig. 3. Differences between control and metal ion treatment groups were not significant. During storage, nitrosylhemochrome content appeared to decrease in all of the groups, and after 1 week, the color faded very rapidly in the cupric ion treatment group.

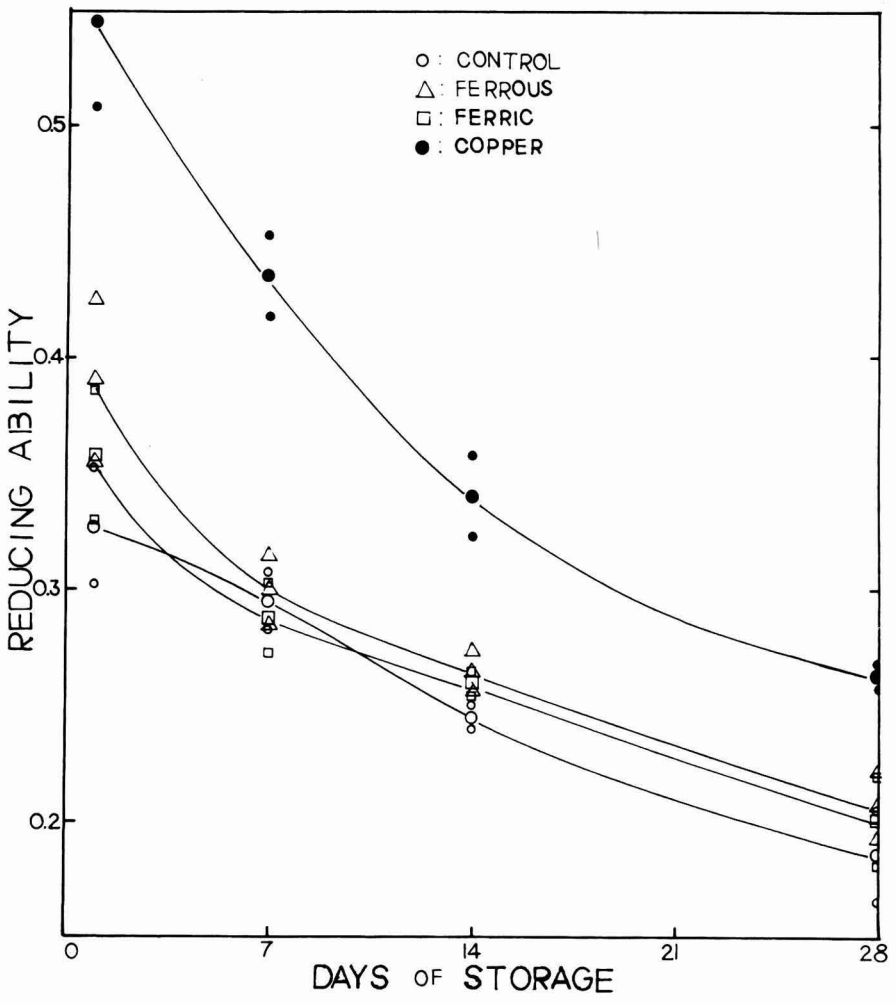

FIG. 2. EFFECT OF METAL IONS ON REDUCING ABILITY IN A MEAT SYSTEM DURING STORAGE

The first measurement was made at one day after mixing and heating. Standard deviations are shown with appropriate symbols above and below the mean.

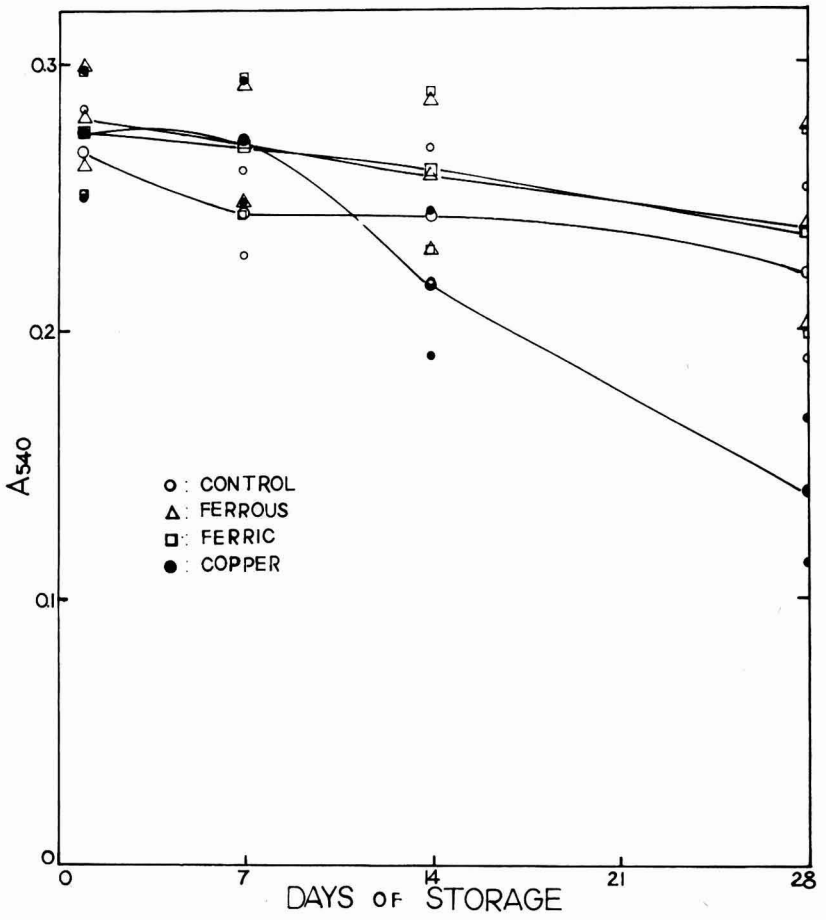

FIG. 3. EFFECT OF METAL IONS ON FORMATION OF NITROSYLHEMOCHROME IN A MEAT SYSTEM

Nitrosylhemochrome content is expressed as absorbance at 540 nm. The first measurement was made at one day after mixing and heating. Standard deviations are shown with the appropriate symbols above and below the mean.

Table 2. Effect of various metal ions at 0.05% on nitrate concentrations in cooked meat systems containing 156 ppm $NaNO_2$ and 1.95% salt[1]

	Treatment			
	Control	Ferrous	Ferric	Cupric
Storage	Nitrate Content in ppm[2] of $NaNO_3$			
1 day	4 ± 1	3 ± 1	3 ± 2	2 ± 1
7 days	3 ± 1	2 ± 1	3 ± 1	4 ± 1
14 days	4 ± 0	4 ± 1	3 ± 1	5 ± 0
28 days	5 ± 1	4 ± 2	5 ± 1	7 ± 2

[1]Control at 2% salt

[2]Results are means of three observations, plus and minus standard deviations

The results for analysis of nitrate are given in Table 2. The values obtained were low, ranging from 2 to 7 ppm, and there were no significant differences between control and treatment groups. The meat blank had less than 1 ppm of nitrate.

During the second phase of the experiments, calcium (Ca^{++}), magnesium (Mg^{++}), and zinc (Zn^{++}) ions were studied. The results for residual nitrite (second phase experiments) are shown in Fig. 4. No differences were observed between the control and the Ca^{++} and Mg^{++} treatment groups, however a significant difference was found between the zinc ion treatment group and the other groups ($P < 0.05$). Table 3 shows that only the zinc ion treatment group had a lower pH, after cooking, than did the control ($P < 0.05$). However, the result in Table 5 shows that the level of residual nitrite in zinc ion treatment was not much different from that of control after pH was adjusted. The calculation of slopes based upon a first order reaction showed

Table 3. Effect of treatment method and metal ion on change in pH of meat systems[1]

Treatment	Control	Calcium	Magnesium	Zinc
	pH Value[2]			
After mixing	$5.58 \pm 0.04^{A,B}$	$5.57 \pm 0.04^{A,B}$	5.59 ± 0.04^{A}	5.51 ± 0.05^{B}
After cooking	5.86 ± 0.05^{A}	5.85 ± 0.04^{A}	5.86 ± 0.05^{A}	5.72 ± 0.04^{B}

[1]Control had 2% NaCl while the others had 1.95% NaCl and 0.05% of metal ions as chloride salts.

[2]Mean of 3 observations ± SD. Values with different superscript letters in the same row are significantly different ($P < 0.05$)

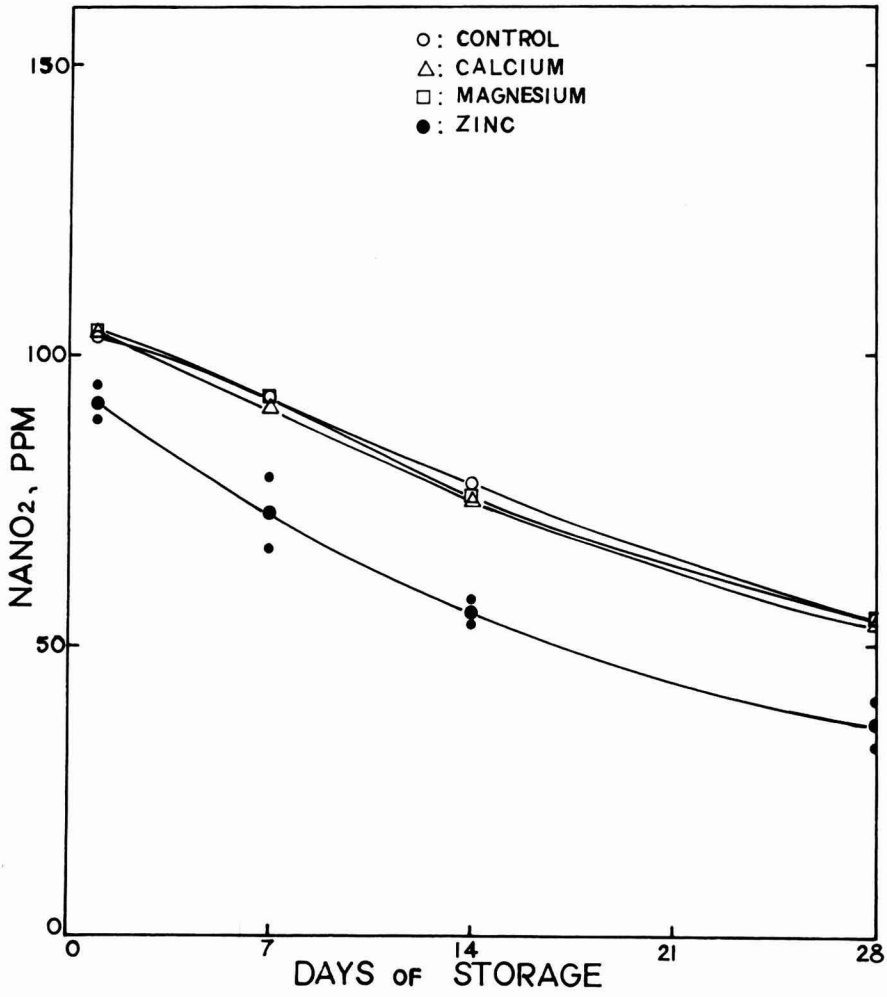

FIG. 4. EFFECT OF METAL IONS ON RESIDUAL NITRITE IN A MEAT SYSTEM

The first measurement was made at one day after mixing and heating. Standard deviations are omitted for control, calcium and magnesium ion treatment groups because variability of data was small.

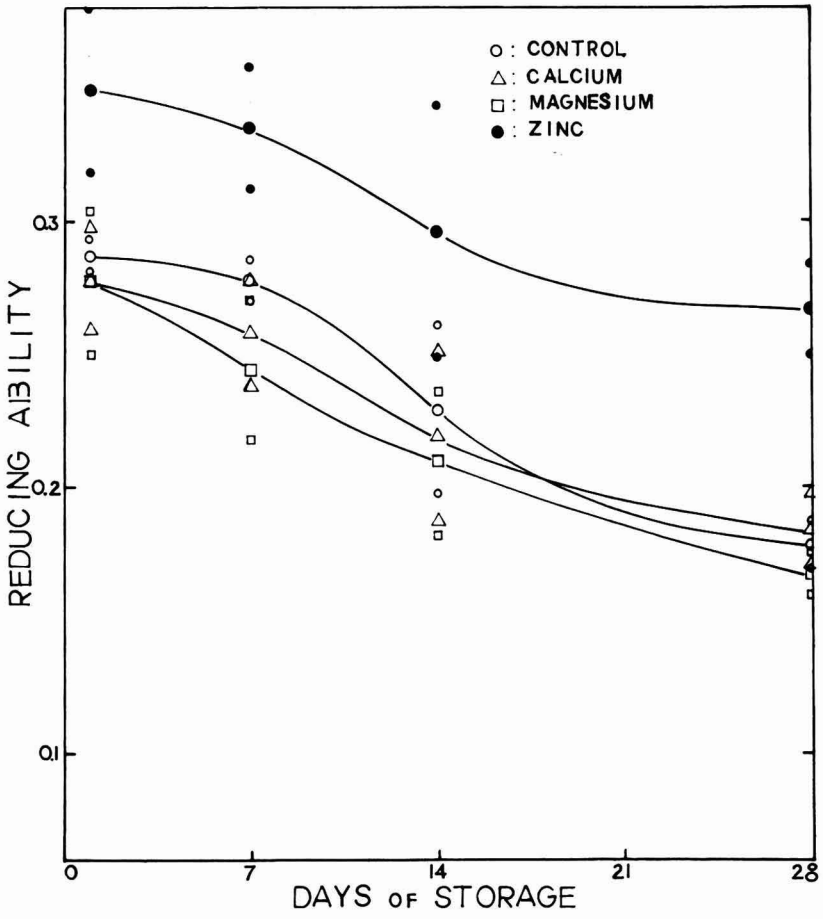

FIG. 5. EFFECT OF METAL IONS ON REDUCING ABILITY IN A MEAT SYSTEM DURING STORAGE

The first measurement was made at one day after mixing and heating. Standard deviations are shown with the appropriate symbols above and below the mean.

that the control had −0.0102, the calcium ion treatment −0.0108, the magnesium ion treatment −0.0106 and the zinc ion treatment group −0.0143. They were not significantly different.

As shown in Fig. 5, only the zinc ion treatment group had a higher reducing ability than the control ($P < 0.05$). However, the result from Table 5 shows that zinc ion itself caused considerable amount of reducing ability. Calcium and magnesium ion treatment groups were not significantly different in reducing ability from the control. During storage, reducing ability

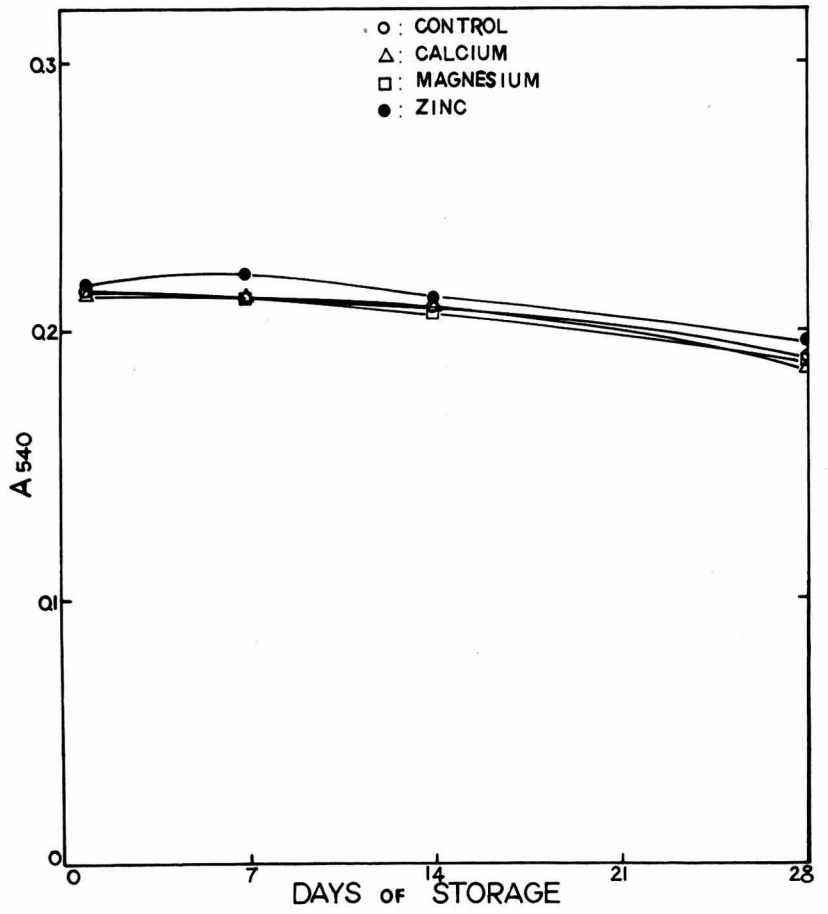

FIG. 6. EFFECT OF METAL IONS ON FORMATION OF NITROSYLHEMOCHROME IN A MEAT SYSTEM

Nitrosylhemochrome content is expressed as absorbance at 540 nm. The first measurement was made at one day after mixing and heating. Standard deviations are omitted for all treatments because variability of data was small.

decreased in all the groups; a similar pattern was observed previously in the first phase of experiments. Differences among control and treatment groups were not significant for nitrosylhemochrome content at any of the storage times examined (Fig. 6). There were no significant differences between control and treatment groups for nitrate (Table 4), all having about 5 ppm.

Table 4. Effect of various metal ions at 0.05% on nitrate concentrations in cooked meat systems containing 156 ppm $NaNO_2$ and 1.95% salt[1]

	Treatment			
	Control	Calcium	Magnesium	Zinc
Storage	Nitrate Content in ppm[2] of $NaNO_3$			
1 day	5 ± 1	5 ± 1	5 ± 1	6 ± 1
7 days	5 ± 1	6 ± 1	5 ± 1	6 ± 1
14 days	6 ± 1	6 ± 1	5 ± 2	6 ± 1
28 days	5 ± 1	5 ± 1	4 ± 1	5 ± 1

[1]Control at 2% salt
[2]Results are means of three observations, plus and minus standard deviations

Table 5. Effects of various metal ions on residual nitrite in the model systems[1]

	Buffer System[2]		Meat Slurry System[4]
Treatment	Residual Nitrite (as % of control)	Reducing Ability[3]	Residual Nitrite (ppm $NaNO_2$)
Control	100	0	143 ± 2
Calcium	100	0	—
Cupric	100	0.14	128 ± 2
Ferrous	96	—	134 ± 1
Ferric	100[5]	0	136 ± 4
Magnesium	100	0	—
Zinc	100	0.06	140 ± 1

[1]Results are expressed as mean values from two observations
[2]The system contained 25 mM PIPES buffer at pH 5.8 and 156 ppm $NaNO_2$ with or without 0.05% metal ions, and was heated at 80° C for 10 min
[3]Absorbance at 420 nm of 1 mM $K_3Fe(CN)_6$ minus absorbance of the solution of 1 mM $K_3Fe(CN)_6$ including 1 ml of sample
[4]The system contained the mixture of 25 mM PIPES buffer 3 : meat 2 (v/w) at pH 5.8 and 156 ppm $NaNO_2$ with or without 0.05% metal ions, and was heated at 80° C for 30 min. Results are expressed as mean ± standard deviation.
[5]At pH 3.0 because ferric ion solubility is extremely low at pH 6 in the absence of ligands (Kragten 1978)

DISCUSSION

Only the ferrous ion caused loss of nitrite in the meat and buffer systems. Ferric, cupric and zinc ions were active in depletion of nitrite only in the system containing meat. In view of the results from the model systems, the effect of metal ions on residual nitrite in a meat system seems to be a complex of the increased reducing ability and decreased pH due to metal ions. Decreased pH appeared to be the major reason for nitrite depletion with the zinc ion treatment group. Therefore, the results suggest that ferric and cupric ions be reduced by endogenous reducing substances, but it is improbable that zinc ion could be so reduced. Olsman (1974) pointed out that pork contains endogenous ascorbic acid. Ando (1974) reported that ferrous ions decomposed nitrite in the absence of ascorbic acid whereas ferric ions behaved similarly to ferrous ions only in the presence of ascorbic acid. However, zinc ion did not show significant activity in nitrite depletion even in the presence of ascorbic acid.

Since degradation of ascorbic acid in food occurs in the presence of cupric or ferric ions, and/or during heating (Tannenbaum 1976), results in Fig. 2 are consistent with the notion that ferrous ions increased reducing ability directly while ferric and cupric ions increased reducing ability only after being reduced by endogenous reducing substances such as ascorbic acid which is degraded due to heating regardless of whether metal ions are present. Moreover, ferric ion did not cause reducing ability in the model system and reducing ability caused by cupric ion in the model system could not account for all of the difference between the cupric ion treatment group and the control in the meat system. The difference in reducing ability between the cupric ion and ferrous or ferric ion treatment groups is difficult to explain because it is difficult to calculate exactly how much reducing ability was induced by reduction of cupric or ferric ion and by ferrous ion itself. Reducing ability caused by zinc ion in the meat system was probably due mainly to zinc ion per se because zinc ion is difficult to reduce in a meat system and the reducing ability caused by zinc ion itself seemed to explain most of that in the meat system. After heating, oxidation predominated over reduction because reducing ability appeared to be decreasing with time of storage in all groups.

If the nitrite depletion due to the addition of metal ions is a reductive process, it would seem logical that more nitric oxide would be produced leading to increased amounts of nitrosylhemochrome. It is well known that ferrous, ferric, cupric and zinc ions enhance the formation of cured color in model systems (Weiss *et al.* 1953; Siedler and Schweigert 1959; Reith and Szakaly 1967). However, results in Fig. 3 and 6 failed to show significant differences in nitrosylhemochrome content. This finding might be due to the fact that we used a meat system while the other investigations involved model

systems containing myoglobin or hemoglobin. Even though we observed some visual differences in cured color intensity between control and ferrous, ferric, cupric and zinc ion treatment groups in samples containing meat, the reason that we failed to have significant differences in results is that there might be some loss of nitrosylhemochrome due to metal ions during extraction. Rapid fading of nitrosylhemochrome in the cupric ion treatment group after one week might be due to a return of the strong oxidizing role of the cupric ion since data in Fig. 2 and Table 2 suggested reoxidation to cupric ion and increasing nitrate formation during storage.

Even though the comparison of slopes between the control and treatments (except the cupric ion treatment) did not show statistically significant difference, if the pH of each treatment group is considered, there should have been differences in the rate of depletion of nitrite during storage (Nordin 1969; Olsman and Krol 1972). However, low storage temperature might have curbed the effect of pH on the depletion of nitrite and a limited number of replication in this experiment might be a possible reason for nonsignificant difference in the statistical analysis. The fact that ferrous, ferric and zinc ions did not result in significantly different depletion rates of nitrite during storage and the fact that no difference in nitrate content was observed suggest that metal ions added to meat do not play any role in depletion of nitrite during storage or that added metal ions cannot have the same effect as the endogeneous metal ions on nitrite depletion during storage. However, cupric ion seemed to behave in a different way because it resulted in a more rapid decrease in nitrite and a tendency for an increase in nitrate during storage. Since the concentrations of metal ions used in this study were far greater than those found in meat, the significance of effects of metal ions added to meat on residual nitrite remains to be considered not in terms of mechanism of loss in residual nitrite but as regards replacement of NaCl or contaminants to NaCl.

REFERENCES

ANDO, N. and NAGATA, Y. 1970. Effects of some fractions of porcine muscle the behavior of nitrite and the formation of cooked cured meat color. 16th European Meeting of Meat Research Workers, pp. 859-878.

ANDO, N., OHASHI, T. and ITO, T. 1973. Some observations on the waterholding capacity of meat. 19th European Congress of Meat Research Workers, pp. 1431-1445.

ANDO, N. 1974. Some compounds influencing color formation. Proc. Int. Symp. Nitrite Meat Prod., Zeist, Pudoc, pp. 143-160.

CHRISTIANSEN, L. N., TOMPKIN, R. B. and SHAPARIS, A. B. 1978. Fate of Clostridium botulinum in perishable canned cured meat at abuse temperature. J. Food Prot. *41*, 354-355.

FOLLETT, M. J. and RATCLIFF, P. W. 1963. Determination of nitrite and nitrate in meat products. J. Sci. Fd. Agri. *14*, 138-144.

FOX, J. B., JR. and NICHOLAS, R. A. 1974. Nitrite in meat. Effect of various compounds on loss of nitrite. J. Agr. Food Chem. *22*, 302-306.

GIBSON, Q. H. 1943. The reduction of methaemoglobin by ascorbic acid. Biochem. J. *37*, 615-618.

HORNSEY, H. C. 1956. The color of cooked cured pork. 1. Estimation of nitric oxidehaem pigments. J. Sci. Fd. Agri. *7*, 534-540.

KRAGTEN, J. 1978. *Atlas of Metal-Ligand Equalibria in Aqueous Solution*, pp. 322-323, John Wiley & Sons, New York.

LEE, M. and CASSENS, R. G. 1980. Effect of sodium chloride on residual nitrite. J. Food Sci. *45*, 267-269.

LEE, S. H. and CASSENS, R. G. 1976. Effect of muscle type on residual nitrite in cured meat. J. Food Sci. *41*, 100-101.

MÖHLER, K. 1971. "Bilanz der Bildung des Pökelfarbstoffs im Muskel fleisch. II. Hitzekatalysierte Bildung des Stickoxydkomplexes." Z. Lebensm. Untersuch. Forsch. *147*, 123-127.

NORDIN, H. R. 1969. The depletion of added sodium nitrite in ham. Can. Inst. Food Technol. J. *2*, 79-85.

OLSMAN, W. J. and KROL, B. 1972. Depletion of nitrite in heated meat products during storage. 18th European Meeting of Meat Res. Workers, pp. 409-415.

OLSMAN, W. J. 1974. About the mechanism of nitrite loss during storage of cooked meat products. Proc. Int. Symp. Nitrite Meat Prod., Zeist, Pudoc, pp. 129-137.

REITH, J. E. and SZAKALY, M. 1967. Formation and stability of nitric oxide myoglobin. I. Studies with model systems. J. Food Sci. *32*, 188-193.

ROSE, D. and PETERSON, R. 1953. Non-bacterial reduction of nitrite in pork. Food Technol. *7*, 369-372.

SEMAN, D. L., OLSON, D. G. and MANDIGO, R. W. 1980. Effect of reduction and partial replacement of sodium on bologna characteristics and acceptability. J. Food Sci. *45*, 1116-1121.

SIEDLER, A. J. and SCHWEIGERT, B. S. 1959. Effects of heat, nitrite level, iron salts, and reducing agents on formation of denatured nitrosomyoglobin. J. Agr. Food Chem. *7*, 271-274.

SNEDECOR, G. W. and COCHRAN, W. G. 1973. *Statistical Methods* (6th ed), pp. 271-275, Iowa State University Press, Iowa.

SWIFT, C. E. and BERMAN, M. D. 1959. Factors affecting the water retention of beef. I. Variations in composition and properties among eight muscles. Food Technol. *13*, 365-370.

TANNENBAUM, S. R. 1976. Vitamins and minerals. In *Principles of Food Science. Part I. Food Chemistry* (O. R. Fennema, ed.), pp. 360-364, Marcel Dekker, Inc.

TOMPKIN, R. B. 1978. The role and mechanism of the inhibition of C. botulinum by nitrite—is a replacement available? Proc. of 31st Annual Recip. Meat Conf., pp. 135-147.

TOMPKIN, R. B., CHRISTIANSEN, L. N. and SHAPARIS, A. B. 1978. The effect of iron on botulinal inhibition in perishable canned cured meat. J. Fd. Technol. *13*, 521-527.

WALTERS, C. L. and TAYLOR, A. McM. 1963. Biochemical properties of pork muscle in relation to curing. Food Technol. *17*, 354-359.

WEISS, T. J., GREEN R. and WATTS, B. M. 1953. Effect of metal ions on the formation of nitric oxide hemoglobin. Food Research *18*, 11-16.

EFFECT OF THAWING AND SUBSEQUENT CHILLING ON THE QUALITY OF PRAWN, *MACROBRACHIUM ROSENBERGII*

W. K. NIP and J. H. MOY

Department of Food Science and Human Nutrition
University of Hawaii
Honolulu, HI 96822

Received for Publication May 10, 1981

ABSTRACT

A study was made on the shelf life of thawed, freshwater prawns. Prawns frozen by various methods were held frozen for 1 month at −18° C, then thawed and held under ice-chilled condition up to 48 h. Their physical, chemical, microbiological and sensory attributes were examined. Results showed that ice-chilling up to 48 h immediately after thawing induced some changes on the peak force/plateau force ratios of the textural profiles, soluble/insoluble collagen ratio and microbial counts. However, no significant differences were observed between the fresh and the treated prawns on texture, flavor and acceptance suggesting that quality of prawns held under ice-chilled condition up to 48 h after thawing was comparable to the fresh ones.

INTRODUCTION

The freshwater prawn *Macrobrachium rosenbergii* has a postharvest ice-chilled/refrigerated shelf life of 3 to 4 days. With the expected increase in freshwater prawn production in Hawaii (Lee 1980), producers have considered freezing them in 1.13 or 2.27 kg (2.5 to 5.0 lb) blocks for institutional use or for export. A problem of this type of packaging at the institutional level is that thawed product may not all be consumed immediately and may deteriorate while held in the refrigerator for 1 to 2 days. Since information on the shelf life of thawed prawns or similar products, e.g., shrimp and lobster, is not readily available, the purpose of this study was to investigate the effect of post-thawing, ice-chilled storage on prawn quality.

MATERIALS AND METHODS

Prawns

Prawns (11 to 18 count/kg) obtained live (1 kg per 8 *l* of pond water) from Aquatic Farms, Kaneohe, Hawaii were immediately killed in ice slurry. The

Journal of Food Processing and Preservation 5 (1981) 207-213.

pincers and legs of 50 kg of prawns were trimmed off within two hours to provide easy packaging.

Sets of four to five prawns were sealed in polyethylene bags and frozen in air at −18°C overnight. Some were also individually frozen by immersing them in −18°C chilled brine for 2 h or dipped in liquid nitrogen for 2 min, and packed 4 to 5 per polyethylene bag. All were stored at −18°C for 1 month before thawing in the bags in 25°C running water for 10-15 min and analyzed immediately or after chilled storage at 0°C for 24 or 48 h. The ice-chilled condition was achieved by storing thawed product in shaved ice in insulated plastic containers kept in a 4°C walk-in refrigerator. Live prawns killed in ice slurry (fresh prawns) served as the control.

Textural Measurement

Fresh or thawed prawns were cooked in boiling water for 3 min. Of the tails that were cut into 5 to 6 sections, a force-distance curve (Szczesniak *et al.* 1978) was plotted for each section with a PEP Texture Tester (Houston, Texas). The ratio of force to shear and force to push through the shear cell (peak force/plateau force ratio of the force distant curve) for each section was recorded and the average peak force/plateau force ratio for each prawn was calculated (Nip and Moy 1979). A decrease in peak force/plateau force indicated softening. Treatment means were based on 8 replicate prawns.

Chemical Analyses

Trimethylamine and sodium chloride (salt) contents were determined according to AOAC procedures (1975). pH was measured with an Orion surface electrode connected to an Orion 407A Specific Ion Meter. Means of 3 or 4 replicates from each treatment were calculated.

The ratio of soluble to insoluble collagen was determined (Woessner 1961; Hill 1966). Collagen content was calculated on the basis of 81.6 mg hydroxyproline residues/1000 amino acid residues in the prawn collagen or 2 mg hydroxyproline/100 mg prawn collagen (Nip *et al.* 1981). One pooled sample from each treatment was analyzed.

Viable Aerobic Bacteria Counts

Aerobic plate counts of homogenates of control or thawed whole prawns in sterile water (10X) were determined in duplicate by placing 0.1 ml of appropriate aqueous dilutions on agar (Difco) plates. Bacterial colonies were counted after incubation for 3 days at 35°C.

Table 1. Peak force/plateau force ratios, percent salt, soluble/insoluble collagen ratios and viable aerobic bacteria of prawns frozen in air (−18°C), brine (−18°C) and liquid nitrogen, thawed, and held at 0°C for 0, 24, and 48 h

Freezing Methods	Holding Time After Thawing (h) Peak Force/Plateau Force Ratio[a]		
	0[b,c]	24[b]	48[c]
Air freezing, −18°C[d,e]	2.14	2.00	2.00
Brine freezing, −18°C[d]	2.76	2.37	2.31
Liquid nitrogen freezing[e]	2.58	2.25	2.28
Control (fresh)	2.47	NA[f]	NA[f]
	Percent Salt[g]		
	0	24	48
Air freezing, −18°C[d]	0.27	0.24	0.25
Brine freezing, −18°C[d,e]	0.41	0.39	0.43
Liquid nitrogen freezing[e]	0.28	0.29	0.31
Control (fresh)	0.22	NA[f]	NA[f]
	Soluble/Insoluble Collagen Ratio[h]		
	0	24	48
Air freezing, −18°C	0.57	1.03	1.35
Brine freezing, −18°C	0.66	1.59	1.43
Liquid nitrogen freezing	0.93	1.09	1.13
Control (fresh)	0.32	NA[f]	NA[f]
	Viable Aerobic Bacteria Count, Log No.[i]		
	0	24	48
Air freezing, −18°C	5.35	5.25	6.30
Brine freezing, −18°C	6.25	6.35	6.30
Liquid nitrogen freezing	NA[f]	NA[f]	NA[f]
Control (fresh)	5.92	5.75	5.72

[a]Values are means of 8 replicates
[b,c,d,e]Variables with the same letters are significantly different at $p \leq 0.01$ level
[f]Not analyzed due to insufficient samples
[g]Values are means of 4 replicates
[h]Each value represents one analysis only, data not statistically analyzed
[i]Values are means of duplicates, data not statistically analyzed

Sensory Evaluations

Whole prawns from each treatment were boiled in water for 3 min and scored by a trained 8-member panel for texture, flavor and overall acceptance. Fresh prawns (control) were obtained, cooked and served on the day of taste panel. Scoring was expressed on the ABC scale (Thompson and Karmas 1963). Data were transformed to a point scale with A (excellent) = 9, B (Good) = 7, C (Fair) = 5, D (Poor) = 3 and E (Unacceptable) = 1. Mean scores from each treatment were used for comparison.

Statistical Analysis

Experimental data were tested for significance by analysis of variance.

RESULTS AND DISCUSSION

Textural Measurements

Table 1 compares the peak force/plateau force ratios of the force-distance curves of the freshly killed and cooked prawns with those of frozen prawns thawed and stored under ice-chilled conditions for 0, 24 and 48 h. There were significant differences at $P \leq 0.01$ in peak force/plateau force ratios between air-freezing and brine-freezing, and between air-freezing and liquid nitrogen freezing. The peak force/plateau force ratios for 24 h and 48 h samples were significantly ($p \leq 0.01$) lower than their 0 h counterparts. These results indicate that thawed prawns will soften gradually especially during the first 24 h under the ice-chilled condition with time, as measured by the PEP Texture Tester.

Chemical Analyses

There was a small but significant increase in sodium chloride (salt) content due to the brine freezing treatment (Table 1). This affirms results of earlier experiments (Nip and Moy 1979).

pH of thawed prawn abdominal (tail) tissue generally remained within the normal range of 7.0 ± 0.2.

Only trace amounts of trimethylamine-nitrogen were detected during the 48 h storage period.

It is apparent that the soluble/insoluble collagen ratio of freshwater prawns varied with time, especially during the first 24 h (Table 1). The data also indicate that freezing might induce an increase in the soluble/insoluble collagen ratio.

Table 2. Sensory evaluation of prawns (8 replicates each) frozen in air (-18°C) brine (-18°C) or liquid nitrogen, thawed and held at 0°C for 0, 24 and 48 h on a 9-point scale.

Freezing Methods	Holding Time After Thawing (h)		
	Texture		
	0[a]	24[a,b]	48[b]
Air freezing, −18°C[c]	7.00	7.87	6.13
Brine freezing, −18°C[c,d]	7.75	7.75	7.87
Liquid nitrogen freezing[d]	7.00	6.75	6.88
Control (fresh)	7.50	NA[e]	NA[e]
	Flavor		
	0	24	48
Air freezing, −18°C	7.25	7.75	6.88
Brine freezing, −18°C	7.50	7.37	7.62
Liquid nitrogen freezing	7.12	6.50	6.88
Control (fresh)	7.25	NA[e]	NA[e]
	Acceptance		
	0[c]	24[c,d]	48[d]
Air freezing, −18°C	7.13	7.83	6.75
Brine freezing, −18°C	7.63	7.63	7.25
Liquid nitrogen freezing	7.00	6.75	6.75
Control (fresh)	7.38	NA[e]	NA[e]

[a,b]Variables with the same letters are significantly different at $p \leq 0.01$ level
[c,d]Variables with the same letters are significantly different at $p \leq 0.05$ level
[e]Not analyzed due to insufficient samples.

Microbiological Assay

Viable aerobic bateria counts in fresh and thawed prawns held at 0°C for 0, 24 and 48 h remained rather constant up to 48 h except for those in the air freezing samples which increased by about 1 log cycle between 24 and 48 h. This was probably due to the longer time it took the prawns to freeze in air, thus allowing the psychrophiles to multiply. High initial counts (10^5 to 10^6) might have been due to the use of whole live prawns and their transportation in pond water.

Sensory Evaluation

Results of sensory evaluations are summarized in Table 2. Prawns frozen in brine exhibited significantly better texture than those frozen by other methods. No significant difference in flavor among various samples were observed. The texture and acceptance of these thawed prawns showed significant differences during the test storage period. However, there was no consistent trend of quality deterioration. Whether this is due to the relatively high rating of the 24 h, air freezing samples is not clear.

No significant differences in texture, flavor and acceptance were observed between the fresh prawns and the thawed and ice-chilled samples. All of the ratings on these experimental samples fell within the A to C+ range, i.e., excellent to fair (Thompson and Karmas 1963). This indicates that the eating qualities of frozen then thawed, prawns held under ice-chilled (0°C) condition up to 48 h are comparable to the fresh ones.

CONCLUSION

Ice-chilled storage of frozen, thawed freshwater prawn for 48 h could induce some changes in their peak force/plateau force ratio, soluble/insoluble collagen ratio and microbial counts. Results of sensory evaluation showed that the eating quality of treated prawns were comparable to that of the fresh ones. It is concluded that freshwater prawns can be frozen by several methods, thawed, and held under ice-chilled conditions up to 48 h with only minimal softening.

ACKNOWLEDGEMENTS

This study was partially sponsored by the University of Hawaii Sea Grant College Program under Institutional Grant No. 04-8-M01-178 from N.O.A.A., Office of Sea Grant, U. S. Dept. of Commerce, The Aquaculture Development Program, Dept. of Land and Natural Resources, State of Hawaii, and the College of Tropical Agriculture and Human Resources, University of Hawaii. University of Hawaii Sea Grant College Program Journal Contribution No. UNIHI-SEAGRANT-JC-81-20 and Hawaii Institute of Tropical Agriculture and Human Resources Journal Series No. 2529.

REFERENCES

A.O.A.C. 1975. Official Methods of Analysis. Association of Official Analytical Chemists. Washington, D.C.

HILL, F. 1966. The solubility of intramuscular collagen in meat animals of various ages. Food Res. *31*, 161-166.

LEE, S. R. 1979. Hawaiian prawn industry, a profile. Aquaculture Development Program, Dept. of Land and Natural Resources, State of Hawaii.

NIP, W. K. and MOY, J. H. 1979. Effect of freezing methods on the quality of prawn, *Macrobrachium rosenbergii*. Proc. World Mariculture Soc. *10*, 761-768.

NIP, W. K., ZEIDAN, H. M. K. and MOY, J. H. 1981. The amino acid profile of insoluble collagen isolated from freshwater prawn *Macrobrachium rosenbergii*. J. Food Sci. *46*, 1633-1639

SZCZESNIAK, A. S., EINSTEIN, M. and PABST, R. E. 1974. The Texture Tester, principles and selected applications. J. Texture Studies *5*, 299-316.

THOMPSON, J. F. and KARMAS, E. 1963. A contribution to quantitative flavor evaluation in product research. Proc. 15th Res. Conf., Council of the Am. Meat Inst. Foundation, University of Chicago, pp. 99-109.

WOESSNER, J. F., JR. 1961. The determination of hydroxyproline in tissue and protein samples containing small protions of this imino acid. Arch. Biochem. Biophys. *93*, 440-447.

RELATIONSHIP AMONG PERCENTAGE OF GRASS GREEN TOMATOES AND COMMINUTED SAMPLE READINGS FOR GRADING PROCESSING TOMATOES

DANIEL LUIS PEDRERO

Iberoamericana University
Mexico City, Mexico

and

PAUL A. CARROAD[1] and MICHAEL O'BRIEN

Department of Agricultural Engineering
University of California
Davis, California 95616

Received for Publication August 20, 1981

ABSTRACT

Over 1000 truck loads of processing tomatoes were studied at a California State grading station to determine the relationships among percentage of grass green tomatoes, Agtron color reading of the comminuted sample, and soluble solids concentration of the comminuted sample. Recorded data were characterized by tomato shape, and with respect to seasonal and time of day variation. No significant correlations were achieved among the variables as currently measured, although general trends of increased redness and solids concentration over the season were as expected.

INTRODUCTION

The economic significance of the processing tomato crop in California has led to codified procedures for grading of loads of tomatoes in order to establish a basis for payment from processor to grower (State of California 1979). Inspectors of the California Department of Food and Agriculture grade tomatoes at the grading stations between harvest and delivery to the processing plant for weight percentage of defects (i.e., tomatoes with worms or mold, grass green tomatoes, material other than tomatoes), color of a comminuted sample, and soluble solids.

[1] Dr. Carroad also is in the Department of Food Science and Technology at the University of California, Davis.

Journal of Food Processing and Preservation 5 (1981) 215-225.

In the grading procedure, a 200-lb sample is obtained from the truckload and is evenly split: 100 lb for manual inspection on a mechanical grading table in which defects are separated as worm and mold infested tomatoes, grass green tomatoes, dirt and extraneous matter, and limited use fruit, and the other 100 lb for preparation of a comminuted sample. Grass green color is defined as the absence of yellow or red coloration on the surface when the tomato is viewed under standardized lighting conditions (Gould 1974). Defects are weighed to the nearest 0.5 lb and expressed directly as percentage values. The accuracy of the subsample being 100 lb is not customarily verified. O'Brien *et al.* (1980) reported that the incoming sample at the grading station averaged 101.7 lb (standard deviation = 6.5 lb).

Of the 100-lb subsample directed for comminution, at least 8.5 lb are selected, the remainder being returned to the truckload. The 8.5-lb sample is washed to eliminate dirt, de-stemmed, and sorted to remove all damaged or grass green tomatoes. The selected fruit is blended under vacuum, so as not to entrain air bubbles which would interfere with the subsequent color reading (Bilton and O'Brien 1976).

From a sieved fraction, eliminating skins and seeds, a sample of juice is taken for a color reading in an instrument such as the Agtron E-5M colorimeter (Magnuson Engineers, Inc.). This instrument is an abridged spectrophotometer with wavelengths of 546 nm and 640 nm whose reading on a 0-100 scale represents a ratio of red to green color (Birth *et al.* 1957). O'Brien *et al.* (1975) stated that an Agtron colorimeter reading of 19 to 36 indicated a fancy color, a reading of 37 to 40 indicated a standard color, and a sample with a color reading above 40 was to be rejected due to a lack of sufficient red color intensity. The comminuted sample is also used for determination of soluble solids content, as measured on a hand-held refractometer.

This paper reports results of a study undertaken to examine the relationships between amount of grass green tomatoes in a load, comminuted sample color reading, and soluble solids content. The grading of tomatoes is a time-consuming operation which subjects sampled fruit to considerable damage and the general load to delay between harvest and processing. If a relationship were established between the variables which constitute the basis of the State grading system, then elimination of grading a well-correlated variable might be possible at a savings in time and labor. This study also examined characteristics of round and pear-shaped tomatoes over early, mid, and late stages of the season to better define changes which occur over time and between tomatoes of different shapes.

METHODS

Data Collection

In 1979 from late July to early October, data were collected on a total of 1,067 truck loads of tomatoes at the Tillie Lewis Grading Station in Woodland, CA.

Information was recorded on date and time of day, shape of tomato as round or pear type, weight of grass green tomatoes in grading sample, and Agtron color reading and soluble solids concentration of the comminuted sample.

The harvesting season was monitored in three periods: early season, July 30 to August 10; mid-season, August 27 to September 5; late season, September 25 to October 4. Daily samples were classified as: morning, before 11:00 AM; noon, from 11:01 AM to 2:00 PM; afternoon, after 2:01 PM.

Grass Green Tomatoes

State grade inspectors determine the weight of grass green tomatoes in the approximately 100-lb sample to the nearest 0.5 lb and express the result as a percentage. In this study, recording of the weight to the nearest ounce was made by a researcher standing next to the inspector.

Comminuted Sample

The Agtron color reading and the soluble solids reading were taken by inspectors and recorded for this study.

Shape

Tomatoes were categorized as round or pear shape by inspection. Specific variety designations were not recorded.

RESULTS AND DISCUSSION

A histogram of the overall data is presented in Fig. 1, showing the number distribution of truck loads categorized by season, time of day, and shape. Round tomatoes accounted for 74.6% of the flow through the grading station and were harvested fairly regularly through the season. Of all round tomatoes, 37.2% were harvested during the early season, 35.4% during mid-season, and 27.4% during late season. In contrast, pear shaped tomatoes showed a peak distribution during mid-season, with 69.0% of the pear shaped tomato harvest appearing during mid-season and only 17.3% and 13.7% being harvested during early and late season, respectively.

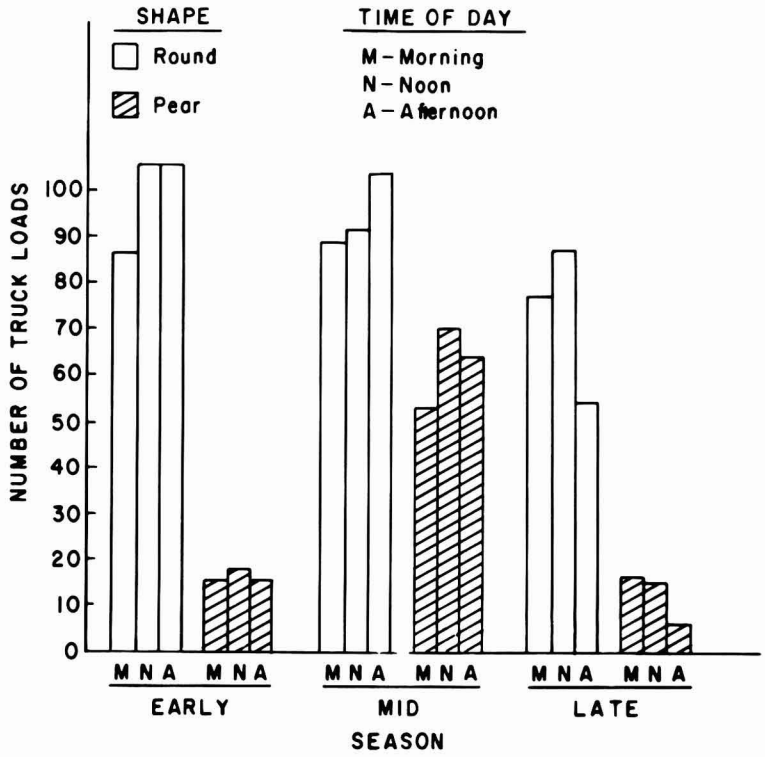

FIG. 1. NUMBER FREQUENCY OF TOMATO TRUCK LOADS SURVEYED ACCORDING TO SHAPE, SEASON, AND TIME OF DAY THE LOAD ARRIVED AT THE GRADING STATION

The weight of grass green tomatoes per 100-lb subsample, Agtron color reading, and soluble solids concentration are listed in Tables 1, 2, and 3, respectively. The number of loads for each shape tomato during the day and over the course of the season is the same for each Table and is listed only in Table 1. In general, there was a tendency for the amount of grass green tomatoes to decrease during the season. For pear shaped tomatoes the main harvesting occurred during the mid-season. Results indicated that 95% of the grass green weight for all loads was below 25 oz, which is well below the 4-lb legal limit. There was an increase over the season of loads with no grass green tomatoes, rising from 4.7% for round shaped and 6.4% for pear-shaped in early season to 11.9% for round shaped and 10.8% for pear-shaped in late season.

Mean Agtron reading for both round and pear shaped tomatoes showed a statistically significant decrease ($p = 0.01$) (i.e., redder color) during the season. As the season advanced, prolonged vine ripening led to a deeper red hue. Another explanation for improved color could be better management of

Table 1. Weight of grass green tomatoes per 100-lb subsamples in truck loads

	Early Season		Mid Season		Late Season	
	Round Shape	Pear Shape	Round Shape	Pear Shape	Round Shape	Pear Shape
Morning						
Number of loads	86	15	88	53	77	16
Mean grass green, ounces	9.5	3.9	9.1	7.9	8.0	9.0
Standard error, %	0.9	0.7	1.0	0.8	1.0	2.8
Noon						
Number of loads	105	17	91	70	87	15
Mean grass green, ounces	11.7	5.0	8.2	6.9	6.0	6.9
Standard error, %	1.1	1.5	0.8	0.8	0.6	1.5
Afternoon						
Number of loads	105	15	103	64	54	6
Mean grass green, ounces	8.8	3.5	11.4	7.4	7.1	2.2
Standard error, %	0.8	0.6	1.0	0.7	0.9	0.4

Table 2. Agtron color readings of comminuted tomato samples

	Early Season		Mid Season		Late Season	
	Round Shape	Pear Shape	Round Shape	Pear Shape	Round Shape	Pear Shape
Morning						
Mean reading	27.6	28.1	26.9	27.3	24.8	23.6
Standard error, %	0.3	0.5	0.4	0.4	0.3	0.5
Noon						
Mean reading	28.4	28.2	27.8	27.2	24.5	24.5
Standard error, %	0.4	0.5	0.4	0.3	0.3	0.8
Afternoon						
Mean reading	28.3	29.0	27.9	27.6	26.9	21.8
Standard error, %	0.3	0.4	0.4	0.3	0.4	0.4

NOTE: Number of loads is as listed in Table 1.

Table 3. Soluble solids concentration of comminuted tomato samples

	Early Season		Mid Season		Late Season	
	Round Shape	Pear Shape	Round Shape	Pear Shape	Round Shape	Pear Shape
Morning						
Mean concentration, %	4.5	4.2	5.1	4.9	5.4	5.3
Standard error, %	0.1	0.1	0.1	0.1	0.1	0.1
Noon						
Mean concentration, %	4.6	4.2	5.2	5.2	5.2	5.2
Standard error, %	0.04	0.1	0.1	0.1	0.1	0.1
Afternoon						
Mean concentration, %	4.6	4.4	5.3	5.2	5.4	5.2
Standard error, %	0.04	0.1	0.1	0.1	0.1	0.1

NOTE: Number of loads is as listed in Table 1.

harvesting. The soluble solids concentration generally increased throughout the season. This result is in accord with the report of Hulme (1970) that the soluble solids concentration of tomatoes increases as the color of the fruit shifts from green to red.

Graphs of number frequency of loads versus weight of grass green tomatoes per 100-lb subsample, Agtron color readings, and soluble solids concentration are shown in Fig. 2, 3 and 4 for round-shaped tomatoes. Results for pear-shaped tomatoes are similar and are not shown. Figure 2 illustrates the tendency for a decrease in percentage of grass green tomatoes as the season progressed. Figures 3 and 4 show the decrease in Agtron readings and the increase in soluble solids concentrations as the season progressed.

The linear correlations among weight of grass green tomatoes, Agtron color readings, and soluble solids concentrations were calculated, and are listed in Table 4. Clearly, no significant linear correlation exists among the measured values. Nor were any significant nonlinear correlations found. Weight of grass green tomatoes were removed before the comminuted samples were prepared. One might hypothesize that the percentage of grass green tomatoes would bear more relationship to Agtron readings if the green tomatoes were included in the comminuted samples. Despite a general tendency for Agtron readings to decrease and soluble solids concentrations to increase over the season as concluded from Table 2 and 3 and Fig. 3 and 4, the paired values of individual loads exhibit no consistent correlation. It is concluded that each of the three measured parameters is statistically independent under current practice and that elimination of one measurement to speed grading would result in loss of information.

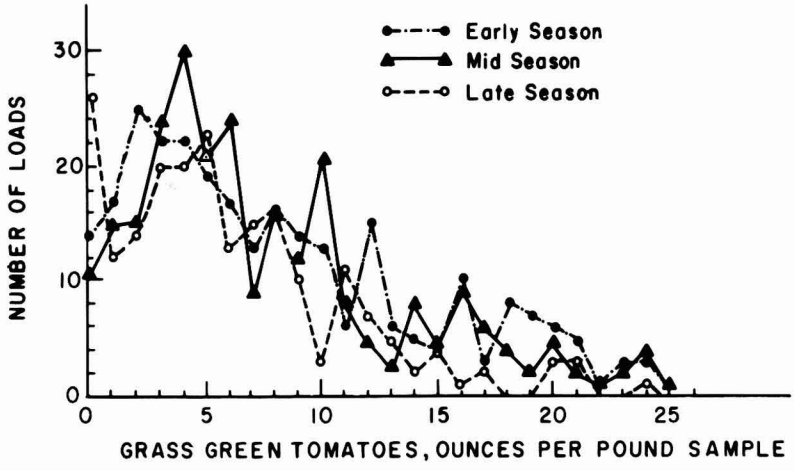

FIG. 2. NUMBER DISTRIBUTION OF LOADS WITH A GIVEN WEIGHT OF GRASS GREEN ROUND-SHAPED TOMATOES PER 100-lb SAMPLE

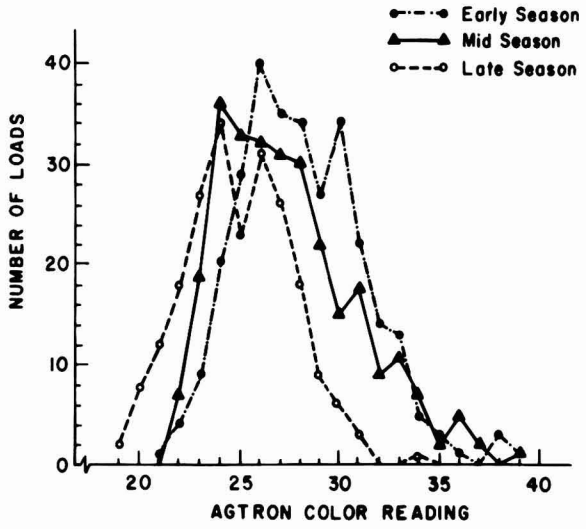

FIG. 3. NUMBER DISTRIBUTION OF LOADS WITH A GIVEN AGTRON COLOR READING OF COMMINUTED SAMPLE OF ROUND-SHAPED TOMATOES

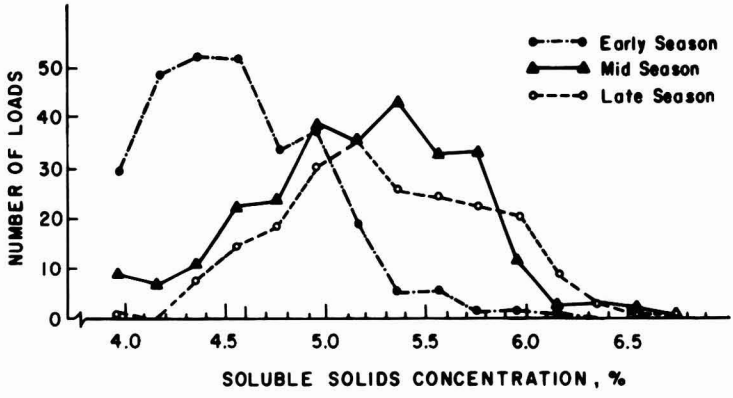

FIG. 4. NUMBER DISTRIBUTION OF LOADS WITH A GIVEN SOLUBLE SOLIDS CONCENTRATION IN COMMINUTED SAMPLE OF ROUND-SHAPED TOMATOES

Soluble solids concentrations shown represent 2-point moving averages to nullify bias of graders to read refractometer scale preferentially at even-numbered scale markings.

Table 4. Correlations between weight of grass green tomatoes (ounces per pound), Agtron color readings, and soluble solids concentrations (percent). Values shown are correlation coefficients (r)

		For Round Shaped Tomatoes			For Pear Shaped Tomatoes		
Correlation	For All Tomatoes	Early Season	Mid Season	Late Season	Early Season	Mid Season	Late Season
Grass green versus agtron reading	0.207	0.078	0.183	0.144	0.087	0.205	0.354
Grass green versus soluble solids	-0.016	-0.003	0.029	0.023	-0.083	0.109	0.121
Agtron reading versus soluble solids	-0.175	0.073	-0.177	0.148	-0.347	-0.064	0.263

ACKNOWLEDGEMENT

This project received partial financial support from the University of California Agricultural Experiment Station, Project 3531-H.

REFERENCES

BILTON, J. E. and O'BRIEN, M. 1976. A vacuum blending method for preparing tomato samples for color determination. Transactions of the ASAE *19*, 386-389.

BIRTH, G. S., NORRIS, K. H. and YEATMAN, J. N. 1957. Nondestructive measurements of internal color of tomatoes by spectral transmission. Fodd Technol. *11*, 552-557.

GOULD, W. A. 1974. *Tomato Production, Processing and Quality Evaluation.* AVI Publishing Co., Westport, Conn.

HULME, A. C. 1970. *The Biochemistry of Fruits and Their Products,* Vol. 1. Academic Press, New York.

O'BRIEN, M., LEONARD, S. J. and MARSH, G. L. 1975. Color determination for loads of processing tomatoes. Transactions of the ASAE *18*(4), 745-747.

O'BRIEN, M., PRUSSIA, S., SMITH, N. E. and ZAHNER, C. 1980. Research Report, 1979. Processing tomato grading equipment. Progress on tomato grading station. Electronic weighing and recording systems. University of California at Davis, Department of Agricultural Engineering.

State of California, California Administrative Code, Register 70, No. 18. May 5, 1979. Title 3: Agriculture, Chapter 3: Economics. Subchapter 1: Gruit and Vegetable Standardization. Group 1: Canning Tomatoes.

THE REMOVAL OF TRIMETHYLAMINE OXIDE AND SOLUBLE PROTEIN FROM INTACT RED HAKE MUSCLE BY WASHING

LORIE A. LANDOLT[1] and HERBERT O. HULTIN
Department of Food Science and Nutrition
Marine Foods Laboratory
University of Massachusetts Marine Station
Gloucester, MA 01930

Received for Publication September 14, 1981

ABSTRACT

Soaking red hake fillets in water (1:20) removes significant amounts of TMAO and water-soluble proteins from the exterior but not interior muscle layers. Scoring the muscle before soaking allowed these components to be removed from the muscle interior. The water uptake which occurred on soaking was increased after scoring. Changing the pH of the soak water had no effect on TMAO or protein removal, but there was inhibition of water uptake at pH 4.0. The addition of NaCl to the extracting solution did not affect TMAO removal but decreased both soluble protein loss and water uptake. The size and shape of the red hake fillets did not materially affect loss of TMAO or soluble protein, but water uptake was related to the surface area to volume ratio. A short-term wash or spray treatment also reduced TMAO and soluble protein contents in the exterior layers of the fillet.

INTRODUCTION

Red hake, *Urophycis chuss*, is a marine fish of the Gadidae family that is widely found along the Eastern coast of the U.S. This species has a white to pinkish-white flesh with a relatively soft texture, as compared to other gadoids, i.e., cod, haddock and cusk. During commercial frozen storage, red hake flesh undergoes a relatively rapid toughening. Dyer and Hiltz (1974) estimated that red hake has a frozen storage life of 22 weeks at -22°C, 5 weeks at -10°C and 2 weeks at -5°C.

The undesirable textural change is believed to be a result of an endogenous enzyme system found in gadoid species that is active at temperatures between 0°C and -26°C (Castell *et al.* 1973; Hiltz *et al.* 1976). This enzyme system converts trimethylamine oxide (TMAO) to dimethylamine (DMA) and formaldehyde. The formaldehyde produced may react with a number of

1 Present address: Ralston Purina Company, 4 RN, Checkerboard Square, St. Louis, MO 63188.

Journal of Food Processing and Preservation 5 (1981) 227-242.

amino acid residues in the fish muscle to form an extensive network of methylene crosslinkages (Fraenkel-Conrat and Olcott 1948a, 1948b). Red hake flesh contains 2 to 3 times more TMAO than most other gadoids (Dingle and Hines 1975); this may be part of the reason why this species is more unstable at frozen temperatures than other gadoids (Castell *et al.* 1971).

In the production of surimi, a semi-processed, wet fish protein product (Miyauchi *et al.* 1973), minced Alaska pollock flesh is washed in a chilled-water soaking operation to remove undesirable soluble muscle components, i.e., TMAO, soluble protein, blood and flesh pigments. It was found that TMAO and soluble protein could be removed from minced red hake flesh by a soaking treatment and that the formation of DMA and formaldehyde and textural change were inhibited in this washed muscle during frozen storage (Landolt 1981). However, minced fish products currently have a very limited market in the United States.

The purpose of the experiments presented in this paper was to investigate the efficacy of a soaking treatment on the removal of TMAO and soluble protein from red hake fillets.

MATERIALS AND METHODS

Raw Material

Fresh red hake (*Urophycis chuss*) weighing approximately 2 to 3 lb each were obtained from a fish distributor in Gloucester, Massachusetts and were stored on ice until used.

Whole fillets were cut from the fish and skinned with as little damage to the muscle as possible. Unless indicated otherwise, pieces of muscle were cut from the front, thicker section of the fillets and included the central line of red muscle. The dimensions of the muscle pieces were: 9.0 cm long, with respect to the frontal/caudal axis of the fish body; 4.0 cm wide, with respect to the dorsal/ventral axis of the fish body; 1.0 cm thick.

Soaking Treatment

A 20:1 soaking procedure was used in all experiments. A pre-weighed piece of muscle was placed in a volume of distilled water equivalent to 20 times the weight of the sample. The water was stirred at a constant, moderate speed and the sample was held off the bottom of the container and protected from the stirring bar by a wire grating. After the soaking period was over, the sample was patted dry with a paper towl so that it was slightly sticky to the touch. The sample was reweighed and stored on ice until used. All soaking treatments were carried out at ambient temperature.

Exterior and Interior Muscle Layers

To prepare exterior and interior muscle layers, the samples were patted dry, immediately placed at -90°C, frozen solid, and held for approximately 4 to 5 h. The exterior muscle layers, each equalling one quarter of the total thickness of the sample, were sliced from the sample and combined for analysis. The interior muscle layer was analyzed as a separate sample from the exterior layers.

Scoring Technique

The scoring technique used on the intact samples involved making slits, approximately 3 mm deep, with a sharp knife on both the skin and skeletal surfaces of the sample. The slits were spaced about 1 cm apart and were made along both the frontal/caudal and dorsal/ventral axes of the sample. They were placed so that those on the skin surface were between those on the skeletal surface. Scoring was performed on the samples prior to the soaking treatment.

Chemical Analyses

The TMAO content of the samples was determined by difference after the TMAO had been reduced to trimethylamine (TMA) by the method described by Yamagata *et al.* (1969). One modification of this method was made: a 2% $TiCl_3$ solution was used as the reducing agent instead of the recommended 1% solution. A 5% trichloroacetic acid extract of fish muscle (Dyer and Mounsey 1945) was used in this procedure. TMA was determined by the picrate salt colorimetric method of Dyer (1945), as modified by Murray and Gibson (1972).

Water-soluble protein extracts were prepared using a phosphate buffer (pH 7.5 and ionic strength of 0.05) (Connell 1960). The protein content of the extracts was determined by a modification of the Lowry procedure (Lowry *et al.* 1951), as described by Markwell *et al.* (1978). A standard curve was prepared using bovine serum album (BSA).

The moisture content of the muscle samples was determined by drying the samples at 110°C for at least 24 h (Dingle and Hines 1975) and was reported on a weight loss percentage basis.

Treatment of Data

"Initial weight" concentration values were obtained by multiplying the concentration of any component of a given weight of soaked muscle by a weight correction factor, the weight ratio. This was defined as the final weight of the soaked muscle sample divided by the initial, unsoaked weight of the same sample.

TMAO and soluble protein losses were determined by subtracting the amount of that component in the soaked sample, calculated on an "initial weight" basis, from the amount present in an unsoaked sample.

Water uptake calculations were made using the following formula: gm H_2O gained per 100 gm initial weight = (100 × weight ratio) (% H_2O is soaked muscle/100) − 82.0. There was an average of 82.0% water in fresh, unsoaked red hake muscle.

Diffusion Coefficient

Diffusion coefficients for TMAO and soluble protein out of red hake muscle during the soaking treatment were determined using a plane sheet model as described by Crank (1970). The experimental method used to generate the data for use in the calculations given in Crank (1970) was based on the loss of solutes from a muscle sample during a soaking treatment; this is a modification of the "total salt uptake" method used by DelValle and Nickerson (1967) to determine diffusion coefficients for salt in salted swordfish.

Statistical Analysis of Data

Differences were determined by Ducan's Multiple Range Test at a probability level of 5% (Steel and Torrie 1960).

RESULTS AND DISCUSSION

TMAO and Soluble Protein Removal in a 20:1 Water Soak

The effects of soaking pieces of red hake muscle in distilled water for soak times up to 4 h on TMAO and soluble protein removal and water uptake are illustrated in Fig. 1. There was an initial fast linear rate of solute removal during the first 30 min of soaking followed by a slower rate of removal. This 2-stage phenomenon may have been due to two types of mass transport: initially, profusion of the components from injured muscle fibers and from extra-cellular spaces, and thereafter, diffusion of the components from the intact muscle fibers.

Diffusion coefficients for TMAO and soluble protein for the 2 stages of removal from the red hake muscle during the 20:1 soaking treatment are given in Table 1. There was a higher diffusion coefficient for TMAO than soluble protein in the first stage of removal, but no difference between the two was observed for the second stage.

TMAO Removal from Muscle Layers During Soak

The TMAO contents of the exterior and interior muscle layers of red hake muscle soaked for 1 and 2 h in distilled water are given in Table 2. There

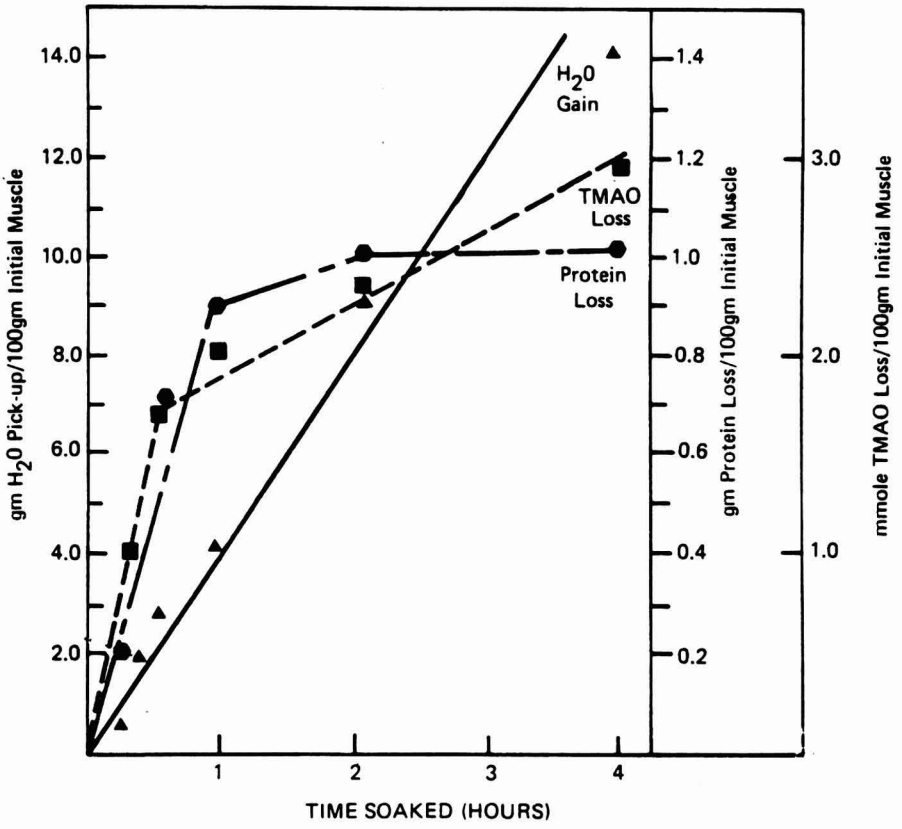

FIG. 1. SOLUTE LOSSES AND WATER UPTAKE IN SOAKED RED HAKE MUSCLE
The samples were prepared and analyzed as described in the Materials and Methods section.

Table 1. Diffusion coefficients for TMAO and soluble protein out of red hake fillet muscle ($\times 10_8$ cm_2/s)

Stage of Removal	D_{TMAO}	$D_{Soluble\ Protein}$
I	2.0	1.2
II	0.5	0.5

Results are averages of 4-6 experiments

Table 2. TMAO contents of exterior and interior muscle layers of soaked and unsoaked red hake muscle pieces (mmole/100 gm initial weight)

Treatment	Exterior Layers	Interior Layers
Control (unsoaked)	$^{a}7.69 \pm 0.70$	$^{a}8.42 \pm 0.59$
Soaked for 1 h	$^{b}5.02 \pm 0.26$	$^{a}7.78 \pm 0.63$
Soaked for 2 h	$^{b}4.90 \pm 0.33$	$^{a}8.20 \pm 0.62$

^{a,b} Values with similar superscripts were not significantly different.
Results are the average of 4 samples

were no significant differences between the two layers in the unsoaked samples. It was observed that significant amounts of TMAO had been removed from the exterior muscle layers, but not from the interior layers. There was no significant difference between the two soak times with respect to TMAO removal.

TMAO Removal from Head Versus Tail Section of Fillet

Whole red hake fillets were soaked for 1 h at room temperature in a volume of distilled water equivalent to 20 times the weight of the sample. Three whole fish were used in this experiment. One fillet per fish was used as an unsoaked control and the other fillet was soaked. TMAO contents of the front and tail sections of the sample fillets are presented in Table 3. There was no significant difference in the TMAO contents of the front and tail sections of the unsoaked control fillets. In the soaked fillets the TMAO concentrations were significantly reduced in both fillet sections with significantly more TMAO being removed from the tail section than from the head section. The greater removal from the tail section is probably due to the fact that the the tail section is thinner and thus the diffusional distances are smaller. In addition, because the section is thinner, a greater proportion of the muscle

Table 3. TMAO contents of the front and tail sections of soaked and unsoaked red hake fillets (mmole/100g initial weight)

Treatment	Front	Tail
Control (unsoaked)	$^{a}7.60 \pm 0.63$	$^{a}7.54 \pm 0.44$
Soaked for 1 h	$^{b}6.12 \pm 0.79$	$^{c}4.85 \pm 0.37$

Values with the same superscripts were not significantly different.
Results are the averages of triplicate samples

cells will have been injured during the filleting and skinning operation. If the cell membrane serves to retard the diffusion of TMAO, then the greater percentage of cellular injury in the tail section should produce a greater efflux of this component.

Influence of pH of Soak Water on the Removal of TMAO and Soluble Protein from Red Hake Muscle

The effect of pH of the soak water on the removal of TMAO and soluble protein from red hake muscle was investigated over the pH range of 4.0 to 8.0. The pH of the water was adjusted prior to the soaking treatment and was held constant throughout the entire hour of the treatment. The estimates of water uptake and component losses are presented in Table 4. It was observed that there was no significant differences among the pH values with respect to TMAO and protein removal. However, there appeared to be an inhibition of water uptake at pH 4.0. The pH at which leached muscle components buffered pure distilled water during a control soak was 6.1. Water uptake by the muscle appeared to be the greatest at this pH. Gill *et al.* (1979) found that low pH washes favored removal of TMAO and reduced protein loss and water uptake in minced cod muscle.

Table 4. Estimated water uptake and component losses in red hake fillet pieces soaked 1 h at different pH values (per 100 gm initial weight)

Soak Water pH	Water Uptake (gm)	TMAO Loss[1] (mmole)	Protein Loss[2] (gm)
4.0	1.73^{b}	1.81^{b}	0.9^{b}
5.0	3.65^{c}	1.00^{b}	0.7^{b}
6.0	5.32^{d}	2.07^{b}	0.9^{b}
7.0	$4.48^{c,d}$	1.33^{b}	0.7^{b}
8.0	3.48^{c}	1.53^{b}	0.5^{b}

[1]The initial TMAO content was 7.01 ± 0.76 mmole/100 g

[2]The initial soluble protein content was 3.38 ± 0.25 g/100 gm initial weight

Results are the averages of triplicate samples. Significant differences among the treatments with respect to final component contents are indicated by different letter superscripts. The superscript "a" indicates that there was no significant difference with respect to initial contents

Influence of NaCl on Removal of TMAO and Proteins from Soaked Red Hake Muscle

The effect of using a NaCl solution as the soaking medium on the composition of soaked red hake muscle was investigated for concentrations between 0.0 and 3.0% (Table 5). TMAO removal was not significantly affected by the addition of salt to the soak water. It appeared as though there was some inhibition of protein loss with the use of the NaCl. This was

Table 5. Estimated water uptake and component losses in red hake fillet pieces soaked 1 h in different NaCl concentrations (per 100 gm initial weight)

% NaCl in Soak Water	Water Uptake (gm)	TMAO Loss[1] (mmole)	Protein Loss[2] (gm)
0.0	2.44^{c}	1.85^{b}	0.6^{c}
1.0	0.60^{b}	1.45^{b}	0.3^{b}
2.0	-0.11^{a}	1.59^{b}	-0.1^{a}
3.0	3.88^{d}	1.56^{b}	0.2^{b}

[1]The initial TMAO content was 7.71 ± 0.79 mmole/100 gm initial weight

[2]The initial soluble protein content was 3.08 ± 0.24 gm/100 gm initial weight

Results are the averages of triplicate samples. Significant differences among the treatments with respect to final component contents are indicated by different letter superscripts. The superscript "a" indicates that there was no significant difference with respect to initial content.

particularly apparent, and statistically significant, at the 2% salt level. A concentration of 1% NaCl in the soak water also decreased the amount of water that was taken up by the soaked red hake muscle. Although it did not affect the removal of TMAO, the use of salt in the soaking water could be used to control water uptake and protein loss. Gill *et al.* (1979) found no significant effect of ionic strength (up to 0.25 M) on TMAO or protein loss or water uptake in minced cod flesh.

Influence of 50% Glycerol on Removal of TMAO and Proteins from Red Hake Muscle

Pieces of red hake fillets were soaked in a 50% glycerol solution equivalent to 5 times the weight of the sample. No differences were observed in the rate

of removal of TMAO and water-soluble proteins from these samples compared to those which were soaked in water when calculated on the basis of the initial weight of the sample. There was, of course, a considerable loss of water in the glycerol-treated samples.

Effect of Scoring Prior to Soaking on Solute Removal from Red Hake Muscle

Scoring of red hake fillets was done to increase the amount of exposed surface area of the samples. The results of an experiment comparing the effects of a 1 h, 20:1 water soaking treatment on muscle samples with and without scoring are given in Table 6. The scoring treatment increased water uptake. Scoring did not increase the amount of protein leached. The data presented

Table 6. Estimates of water uptake and component losses in scored and unscored red hake fillet pieces soaked for 1 h (per 100 gm initial weight)

Treatment	Water Uptake (gm)	TMAO Loss[1] (mmole)	Protein Loss[2] (gm)
Unscored	4.67^{b}	1.98^{b}	0.5^{b}
Scored	6.15^{c}	2.90^{c}	0.8^{b}

[1]The initial TMAO conent was 7.59 ± 0.18 mmole/100 gm initial weight
[2]The initial soluble protein content was 2.78 ± 0.23 gm/100 gm initial weight

Results are the averages of four samples. Significant differences between the treatments with respect to final component contents are indicated by different letter superscripts. The superscript "a" indicates that there was no significant differences with respect to initial contents.

Table 7. TMAO contents of the muscle layers of scored and unscored red hake fillet pieces at various soak times (mmole/100 gm initial weight)

Muscle Layer		Soak Times (min)		
		0	30	60
Unscored:	Exterior	$^{a}7.74 \pm 0.61$	$^{a}4.76 \pm 0.53$	$^{b}5.69 \pm 0.90$
	Interior	$^{a}8.03 \pm 0.40$	$^{c}8.24 \pm 0.43$	$^{c}8.36 \pm 0.21$
Scored:	Exterior	$^{a}7.81 \pm 0.41$	$^{a}4.88 \pm 0.86$	$^{a}3.62 \pm 0.36$
	Interior	$^{a}7.90 \pm 0.47$	$^{b}7.75 \pm 1.02$	$^{b}6.03 \pm 0.19$

[a,b,c]Values in same column with same superscripts were not significantly different
Results are the averages of 4 samples

in Table 7 compare the response of interior and exterior layers to scoring. TMAO was removed in significant amounts from both the exterior and interior muscle layers in the scored samples after 30 and 60 min of soaking. More TMAO was removed from the exterior than the interior layers in the scored samples. In the unscored muscle samples, TMAO was only removed from the exterior layers after these soak times.

The pattern of TMAO removal as a function of time in scored and unscored red hake muscle was different. The scored sample had a more rapid initial rate of removal (first stage) than did the unscored, and it entered the second stage earlier than did the unscored. The rate of TMAO removal in both stages was faster for the scored sample than for the unscored.

Short-Time Washing Procedures

The soaking treatments that have been discussed so far are too long for practical large scale industrial applications. Therefore we looked at procedures which would be more consistent with commercial use. These included short soaking treatments (1 and 5 min) and even shorter spraying treatments (10 and 30 s) (Table 8). It was assumed that the solute content of the interior layers was not significantly different from the initial content of the solutes in muscle (see Table 2). There was a significantly lower TMAO content in the exterior layer compared to the interior layer after soaking for either 1 or 5 min, and there was no advantage of soaking for 5 min compared to 1 min. This short soaking period did not, however, remove a significant amount of water-soluble protein from the red hake fillet.

Table 8. TMAO and water-soluble protein contents of muscle layers in red hake fillets soaked or sprayed for short times

Soak Time	TMAO (mmole/100 g Initial wt)		Water-Soluble Protein (mg/g Initial wt)	
(min)	Exterior	Interior	Exterior	Interior
1	$^{a}6.07 \pm 0.16$	$^{b}7.12 \pm 0.04$	$^{a}23.1 \pm 1.7$	$^{a}25.1 \pm 3.5$
2	$^{a}6.01 \pm 0.01$	$^{b}7.09 \pm 0.01$	$^{a}22.3 \pm 2.9$	$^{a}26.3 \pm 2.2$
^{a,b}Similar superscripts indicate no statistical difference ($p < 0.05$)				
Spray Time				
10	$^{c}5.67 \pm 0.29$	$^{d}8.39 \pm 0.12$	$^{c}20.3 \pm 0.8$	$^{d}24.9 \pm 0.3$
30	$^{c}6.14 \pm 0.33$	$^{d}8.44 \pm 0.23$	$^{c}18.9 \pm 0.7$	$^{d}25.4 \pm 0.4$

^{c,d}Similar superscripts indicate no statistical difference ($p < 0.05$)

Results are the average of triplicate samples

Spraying times of either 10 or 30 s removed a significant amount of both TMAO and water-soluble protein from the hake fillets. Thus, it can be seen that short-term washing steps can be effective in reducing the concentrations of TMAO and water-soluble proteins from the exterior muscle layers of red hake fillets. In addition, the spraying treatment appeared to be somewhat more effective than the short period of soaking.

Geometric Considerations of the Muscle Sample

Diffusion processes are dependent on both the size and shape of the material which is being extracted. There is also the possibility that the extent of cellular injury may affect the rate of diffusion if the cellular membrane limits diffusion of any of the solutes. The more cells that are injured, the greater the liklihood that solutes present in the cells would be able to freely diffuse into the extracting medium. We studied these aspects in the red hake system by using pieces of fillet with different geometries. TMAO and water-soluble protein removal from pieces of red hake muscle cut to various shapes and sizes were compared. In addition, estimates were made of the percentages of muscle fibers that were injured with each shape and size of fish muscle. The assumptions and calculations used in estimating the percentage of muscle fiber injury are discussed in the Appendix.

Some characteristics of the three different sized muscle pieces, along with the data on TMAO removal and water uptake, are given in Table 9. Under our conditions, no effect was observed in the loss of TMAO. (In another set of experiments, no difference was observed in the loss of soluble protein either.) There did appear to be a correspondence between water uptake and

Table 9. Effect of size and shape of muscle on TMAO removal and water uptake

Dimensions[1] (cm)	SA/V[2] (cm^{-1})	Estimated % Fiber Injury[3]	TMAO Loss[4] mmole/100 g Initial wt	Water Gain g/100 g Initial wt
7.0 × 7.0 × 1.2	2.24	24.4—31.0	1.76[b]	0.59[b]
4.0 × 4.0 × 0.9	3.22	42.7—52.7	1.96[b]	4.05[d]
8.0 × 3.0 × 1.1	2.74	22.5—30.0	1.65[b]	1.35[b]

[1]Dimensions are given with respect to head-to-tail axis, dorsal-ventral axis, and thickness, respectively

[2]SA/V = surface area to volume ratio

[3]See Appendix for calculations

[4]The initial TMAO content was 8.21 ± 1.18 mmole/100 gm initial tissue

Results are the averages of duplicate samples. Significant differences between treatments with respect to final component contents are indicated by different letter superscripts. The superscript "a" indicates there was no significant differences with respect to initial contents

the surface area to volume ratio but not to the percentage of injured fibers. Within the range of the sample sizes and shapes used in these experiments, destruction of the cellular membrane (the definition of percent of fiber injury) did not have a significant effect on loss of TMAO. Differences might have shown up over a wider range of sample dimensions, but this was difficult to achieve with the red hake fillets since the 1.2 cm depth represented close to a maximum that could be obtained for this species. These results indicate that with death of the fish, there is a loss of selective permeability of the cell membrane, and it does not retard the movement of molecules like TMAO (and perhaps soluble proteins as well).

APPENDIX

Percent Muscle Fiber Injury Calculations

The percentage of muscle fibers injured in a given piece of red hake fillet was estimated based on the assumptions and calculations presented in this appendix.

Red Hake Musculature

Red hake, a teleost fish, contains a segmented myotomal musculature. The myotomes are wide "W"-shaped, membrane enclosed units that are arranged along the long axis of the fish body (Bone 1978). Each myotome is made up of individual muscle fibers that are arranged at right angles to the myotomal membrane. The individual fibers extend across the entire width of the myotome so that each fiber is inserted in the myotomal membrane (Alexander 1969). The fibers are oriented at between 0° and 40° angles to the long axis of the fish body, depending on their position in the myotome.

Assumption Made

To calculate muscle fiber injury, the following assumptions were made about the fish muscle:

1. All muscle fibers were oriented at 20° from the frontal/caudal axis of the fillet (the long axis of the fish body).
2. Red hake muscle consists primarily of white fibers.
3. The white muscle fibers are right circular cylinders. The diameter was taken to equal 60×10^{-4} cm (Nag 1972). The length of the fibers was taken to be between 0.75 cm and 1.0 cm, based on actual measurements of the myotome width in red hake fillets.

Method of Calculating Muscle Fiber Injury

The steps involved in estimating the percentage of muscle fiber injury in a given piece of red hake due to trimming, shaping and treatment are listed below:

1. Determine the volume of the given piece of muscle.
2. Determine the total number of fibers in the muscle piece by dividing the total volume of the muscle piece by the volume of a single muscle fiber.
3. Determine the total number of fibers injured at the skin and skeletal surfaces, the sides and ends of the muscle piece.
4. Divide the total number of fibers injured by the total number of fibers in the muscle piece and multiply by 100 to obtain a percentage of muscle fiber injury.

Sample Calculation

An example of the types of calculations used in this method of estimating the percentage of muscle fiber injury in a piece of red hake muscle is given below. These calculations are based on a piece of red hake muscle of dimensions 9.0 cm (frontal/caudal axis) by 4.0 cm (dorsal/ventral axis) by 1.0 cm (fillet thickness).

$$\text{Total volume of muscle fiber} = V_{\textit{right circular cylinder}} = \pi r^2 h$$

where r = the radius of the muscle fiber and h = the length of the muscle fiber

For h = 1.0 cm, the total volume of the muscle fiber equals 2.8×10^{-5} cm^3. Total volume of the muscle piece = 9.0 cm × 4.0 cm × 1.0 cm × 36.0 cm^3.

Total number of fibers in muscle piece = 1.3×10^6 fibers.

The number of muscle fibers injured at the ends of the piece is determined by assuming that there are only 9 myotomal lengths in the given piece and that all fibers in two of these myotomes are severed:

$$2/9\ (1.3 \times 10^6 \text{ fibers in total piece}) = 2.9 \times 10^5 \text{ injured fibers.}$$

The number of additional muscle fibers injured at the skin and skeletal surfaces is determined by assuming that only one layer of muscle fibers is damaged on each surface. The packing of the fibers is such that the contribution of each single fiber to the total area on these surfaces is less than would be expected, based on the diameter of the fiber. It is assumed that a good estimate of the contributing area of each fiber can be made by assuming that on the average the distance across the circular surface of the fibers is what is obtained at one-half the diameter from the center point. A factor of 7/9 is also required in the calculation to account for those fibers already damaged at the end surfaces. The formula used is:

$$\frac{7 \times 2(9.0\text{ cm} \times 4.0\text{ cm})}{9 \times (52.0 \times 10^{-4}\text{ cm} \times 1.0\text{ cm})/\text{fiber}} = 1.1 \times 10^4 \text{ injured fibers}$$

The number of additional muscle fibers injured at the side surfaces is calculated based on the assumption that the muscle fibers are oriented at 20° from the long axis of the fillet. Upon cutting the fillet exactly parallel to the long axis, the exposed surfaces of the severed fibers are elliptical in shape. The ellipse representing the exposed surface of a single muscle fiber has a short axis, x, equal to the diameter of the fiber, and a long axis, y, equal to 60×10^{-4} cm/sin 20° = 175.4×10^{-4}cm.

$$\begin{aligned}\text{exposed surface of fiber} &= \pi xy = \pi(175.4 \times 10^{-4}\text{cm})(60 \times 10^{-4}\text{cm}) \\ &= 3.3 \times 10^{-4}\text{cm}^2.\end{aligned}$$

The formula used to determine the number of addition fiber injured at the sides is:

$$\frac{7 \times 2(4.0\text{ cm} \times 1.0\text{ cm})}{9 \times 3.3 \times 10^{-4}\text{cm}^2/\text{fiber}} = 1.9 \times 10^4 \text{ injured fibers.}$$

Total number of muscle fibers injured = 3.2×10^5 fibers.

$$\%\text{ muscle fiber injury} = \frac{3.2 \times 10^5 \text{ injured fibers}}{1.3 \times 10^6 \text{ total fibers}}(100)$$

$$= 24.6\%$$

The percentage of muscle fiber injury in the red hake muscle pieces used in this research were reported as ranges. This was because the myotomal widths ranged between 0.75 and 1.0 cm in the same sample. The fiber length is an important variable in the calculation used to arrive at the injury estimations.

ACKNOWLEDGMENTS

This work was supported in part by NOAA and the New England Fisheries Development Task Force, by the Massachusetts Agricultural Experiment Station, and by the Graduate School of the University of Massachusetts at Amherst.

REFERENCES

ALEXANDER, R. M. 1969. The orientation of muscle fibres in the myomers of fishes. J. Mar. Biol. Assn. U.K. *49*, 263-290.

BONE, Q. 1978. Locomotor muscle. In *Fish Physiology*, Vol. 6, (W. S. Hoar and D. J. Randall, eds. pp. 361-424. Academic Press, New York.

CASTELL, C. H., SMITH, B. and DYER, W. J. 1973. Effects of formaldehyde on salt extractable proteins of gadoid muscle. J. Fish. Res. Bd. Can. *30*, 1205-1213.

CASTELL, C. H., SMITH, B. and NEAL, W. 1971. Production of dimethylamine in muscle of several species of gadoid fish during frozen storage, especially in relation to dark muscle. J. Fish. Res. Bd. Can. *28*, 1-5.

CRANK, J. 1970. *The Mathematics of Diffusion*, Clarendon Press, Oxford.

CONNELL, J. J. 1960. Changes in the actin of cod flesh during storage at -14°C. J. Sci. Food Agric. *11*, 515-519.

DELVALLE, F. R. and NICKERSON, J. T. R. 1967. Studies on salting and drying fish. II. Dynamic aspects of the salting of fish. J. Food Sci. *32*, 218-224.

DINGLE, J. R. and HINES, J. A. 1975. Protein instability in minced flesh from fillets and frames of several commercial Atlantic fishes during storage at -5°C. J. Fish. Res. Bd. Can. *32*, 775-783.

DYER, W. J. 1945. Amines in fish muscle. I. Colorimetric determination of trimethylamine as the picric salt. J. Fish. Res. Bd. Can. *6*, 351-358.

DYER, W. J. and HILTZ, D. F. 1974. Sensitivity of hake muscle to frozen storage. Environ. Can. Fish. & Mar. Ser. News Series Circ. *45*, May 3, 1974.

DYER, W. J. and MOUNSEY, Y. A. 1945. Amines in fish muscle. II. Development of trimethylamine and other amines. J. Fish. Res. Bd. Can. *6*, 359-367.

FRAENKEL-CONRAT, H. and OLCOTT, H. S. 1948a. The reaction of formaldehyde with proteins. V. Crosslinking between amino and primary amide or guanidyl groups. J. Amer. Chem. Soc. *70*, 2673-2684.

FRAENKEL-CONRAT, H. and OLCOTT, H. S. 1948b. Reaction of formaldehyde with proteins. VI. Crosslinking of amino groups with phenol, imidazole or indole groups. J. Biol. Chem. *190*, 827-843.

GILL, T. A., DINGLE, J. R., SMITH-LALL, B. and STANLEY, D. W. 1979. Improved utilization of fish protein—Quality enhancement of mechanically deboned fish. Can. Inst. Food Sci. Technol. J. *12*, 200-202.

HILTZ, D. F., LALL, B. S., LEMON, D. W. and DYER, W. J. 1976. Deteriorative changes during frozen storage in fillets and minced flesh of silver hake processed from round fish held on ice and refrigerated sea water. J. Fish. Res. Bd. Can. *33*, 2560-2567.

LANDOLT, L. A. 1981. Removal of TMAO and soluble protein from red hake muscle by washing: Role in TMAO decomposition during frozen storage. Master's Thesis, University of Massachusetts, Amherst.

LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. J. 1951. Protein measurement with the folin-phenol reagent. J. Biol. Chem. *193*, 265-275.

MARKWELL, M. K., HAAS, S. M., BIEBER, L. L. and TOLBERT, N. E. 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. Anal. Biochem. *87*, 206-210.

MIYAUCHI, D., KUDO, G. and PATASHNIK, M. 1973. Surimi-A semi-processed wet fish protein. Mar. Fish. Rev. *35*(12), 7-9.

MURRAY, C. K. and GIBSON, D. M. 1972. An investigation of the method of determining trimethylamine in fish muscle extracts by the formation of its picric salt. Part I. J. Food Technol. *7*, 35-46.

NAG, A. C. 1977. Ultrastructure and adenosine triphosphatase activity of red and white muscle fibers of the caudal region of a fish, *Salmo gairdneri*. J. Cell Biol. *55*, 42-57.

STEEL, R. G. D. and TORRIE, J. H. 1960. *Principles and Procedures of Statistics*, McGraw-Hill Book Co., Inc., New York.

YAMAGATA, M., HORIMOTO, K. and NAGAOKA, C. 1969. Assessment of green tuna: Determination of trimethylamine oxide and its distribution in tuna muscle. J. Food Sci. *34*, 156-159.

BOOK REVIEW

Enzymes and Food Processing, Edited by G. G. Birch, N. Blakesbrough and K. J. Parker. Applied Science Publishers Ltd., 22 Rippleside Commercial Estate, Ripple Road, Barking Essex IGIIOSA, England. 296 pp. 1981. $44.00.

This book has much to offer for the student and applications technologist who would deal with enzymes in production processing of food. It evolved from an industry/university symposium organized by the National College of Food Technology, University of Reading. Most all major applications of enzyme technology are treated by recognized scientists in the food industry and academia. If I missed any one specific field of coverage it was perhaps in the area of food flavor development. However, chapters range in scope from use of enzymes in fructose manufacture through application of lactases, detoxifying enzymes, and enzymes used in starch syrup technology, in maltose production, fruit juice processing, meat tenderizing, and cheesemaking. A final chapter considers enzymes in analysis of foods. Of particular relevance to the times is a chapter on enzyme hydrolysis in recovery of proteins and modification of proteins to enhance their utility. The reader will find specific examples of applications to animal, plant, and yeast protein systems.

This reviewer found the book to provide enough details to lend insight into theoretical considerations but, equally so, to serve the practical needs of industrial technologists. Flow diagrams, charts, substrate concentrations, pH and temperature optima curves all add to the usefulness of the book. The role of enzyme chemistry in food processing is immediately evident even on casual perusal. More importantly, research thrusts of the future seem to poke out at you at nearly every turn of the page. Work currently underway and that poised for exploitation holds promise of better nutrient utilization, lower cost, and/or greater efficiency in food processing. This book provides a more than adequate glimpse of present possibilities and future prospects. It should indeed serve technologist, student, and researcher.

V.S. PACKARD

AUTHOR INDEX

SUBJECT INDEX

F
N
P

JOURNALS AND BOOKS IN FOOD SCIENCE AND NUTRITION

Journals

JOURNAL OF FOOD SERVICE SYSTEMS, G. E. Livingston and C. M. Chang

JOURNAL OF FOOD BIOCHEMISTRY, H. O. Hultin, N. F. Haard and J. R. Whitaker

JOURNAL OF FOOD PROCESS ENGINEERING, D. R. Heldman

JOURNAL OF FOOD PROCESSING AND PRESERVATION, T. P. Labuza

JOURNAL OF FOOD QUALITY, A. Kramer and M. P. DeFigueiredo

JOURNAL OF FOOD SAFETY, M. Solberg and J. D. Rosen

JOURNAL OF TEXTURE STUDIES, P. Sherman and M. C. Bourne

Books

SHELF-LIFE DATING OF FOODS, T. P. Labuza

ANTINUTRIENTS AND NATURAL TOXICANTS IN FOOD, R. L. Ory

UTILIZATION OF PROTEIN RESOURCES, D. W. Stanley, E. D. Murray and D. H. Lees

FOOD INDUSTRY ENERGY ALTERNATIVES, R. P. Ouellette, N. W. Lord and P. E. Cheremisinoff

VITAMIN B_6: METABOLISM AND ROLE IN GROWTH, G. P. Tryfiates

HUMAN NUTRITION, 3RD ED., R. F. Mottram

DIETARY FIBER: CURRENT DEVELOPMENTS OF IMPORTANCE TO HEALTH, K. W. Heaton

RECENT ADVANCES IN OBESITY RESEARCH II, G. A. Bray

FOOD POISONING AND FOOD HYGIENE, 4TH ED., B. C. Hobbs and R. J. Gilbert

FOOD SCIENCE AND TECHNOLOGY, 3RD ED., M. Pyke

POSTHARVEST BIOLOGY AND BIOTECHNOLOGY, H. O. Hultin and M. Milner

THE SCIENCE OF MEAT AND MEAT PRODUCTS, 2ND ED., J. F. Price and B. S. Schweigert

GUIDE FOR AUTHORS

Typewritten manuscripts in triplicate should be submitted to the editorial office. The typing should be double-spaced throughout with one-inch margins on all sides.

Page one should contain: the title, which should be concise and informative; the complete name(s) of the author(s); affiliation of the author(s); a running title of 40 characters or less; and the name and mail address to whom correspondence should be sent.

Page two should contain an abstract of not more than 150 words. This abstract should be intelligible by itself.

The main text should begin on page three and will ordinarily have the following arrangement:

Introduction: This should be brief and state the reason for the work in relation to the field. It should indicate what new contribution is made by the work described.

Materials and Methods: Enough information should be provided to allow other investigators to repeat the work. Avoid repeating the details of procedures which have already been published elsewhere.

Results: The results should be presented as concisely as possible. Do not use tables *and* figures for presentation of the same data.

Discussion: The discussion section should be used for the interpretation of results. The results should not be repeated.

In some cases it might be desirable to combine results and discussion sections.

References: References should be given in the text by the surname of the authors and the year. *Et al.* should be used in the text when there are more than two authors. All authors should be given in the References section. In the Reference section the references should be listed alphabetically. See below for style to be used.

DEWALD, B., DULANEY, J. T. and TOUSTER, O. 1974. Solubilization and polyacrylamide gel electrophoresis of membrane enzymes with detergents. In *Methods in Enzymology*, Vol. xxxii, (S. Fleischer and L. Packer, eds.) pp. 82–91, Academic Press, New York.

HASSON, E. P. and LATIES, G. G. 1976. Separation and characterization of potato lipid acylhydrolases. Plant Physiol. *57*, 142–147.

ZABORSKY, O. 1973. *Immobilized Enzymes*, pp. 28–46, CRC Press, Cleveland, Ohio.

Journal abbreviations should follow those used in *Chemical Abstracts*. Responsibility for the accuracy of citations rests entirely with the author(s). References to papers in press should indicate the name of the journal and should only be used for papers that have been accepted for publication. Submitted papers should be referred to by such terms as "unpublished observations" or "private communication." However, these last should be used only when absolutely necessary.

Tables should be numbered consecutively with Arabic numerals. The title of the table should appear as below:

Table 1. Activity of potato acyl-hydrolases on neutral lipids, galactolipids, and phospholipids

Description of experimental work or explanation of symbols should go below the table proper.

Figures should be listed in order in the text using Arabic numbers. Figure legends should be typed on a separate page. Figures and tables should be intelligible without reference to the text. Authors should indicate where the tables and figures should be placed in the text. Photographs must be supplied as glossy black and white prints. Line diagrams should be drawn with black waterproof ink on white paper or board. The lettering should be of such a size that it is easily legible after reduction. Each diagram and photograph should be clearly labeled on the reverse side with the name(s) of author(s), and title of paper. When not obvious, each photograph and diagram should be labeled on the back to show the top of the photograph or diagram.

Acknowledgments: Acknowledgments should be listed on a separate page.

Short notes will be published where the information is deemed sufficiently important to warrant rapid publication. The format for short papers may be similar to that for regular papers but more concisely written. Short notes may be of a less general nature and written principally for specialists in the particular area with which the manuscript is dealing. Manuscripts which do not meet the requirement of importance and necessity for rapid publication will, after notification of the author(s), be treated as regular papers. Regular papers may be very short.

Standard nomenclature as used in the scientific literature should be followed. Avoid laboratory jargon. If abbreviations or trade names are used, define the material or compound the first time that it is mentioned.

EDITORIAL OFFICE: Prof. T. P. Labuza, Editor, Journal of Food Processing and Preservation, University of Minnesota, Department of Food Science and Nutrition, Saint Paul, Minnesota 55108 USA

JOURNAL OF FOOD PROCESSING AND PRESERVATION VOL. 5, NO. 4

CONTENTS