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Influence of Freezing Meats on Sausage Fermentation and Drying

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The effects of freezing pork and beef at −17.8 °C up to 90 weeks on fermentation and drying of pepperoni were studied. Standard pork-beef sausage mixtures were made from thawed frozen meat after removal of meat samples from frozen storage at predetermined time periods. These mixtures were fermented 24 hours at 35 °C with added starter culture and then dried 6 weeks at 12 °C and 65% relative humidity. Fermentation patterns (pH and titratable acidity changes) and drying kinetics, including % yield, remained constant in dry sausage prepared from meats frozen up to 90 weeks. Residual nitrite after fermentation and drying was essentially zero during the course of the study and no nitrosamines were formed. Sensory panel evaluation indicated flavor loss in pepperoni prepared from meats frozen 90 weeks at −17.8 °C and/or spices held 90 weeks at 5 °C. These studies indicated that, except for flavor loss, extended frozen storage of pork and beef is not detrimental to dry sausage processing.

Introduction

Drying is the terminal processing operation in the production of semidry and fully dry fermented sausages such as pepperoni, Genoa salami, and summer sausage. In order to eliminate trichinae from pork-containing sausage products, Food Safety and Quality Service (FSQS) rigorously controls the temperature of the dry room and the length of product drying. Research into the effect of variables on the drying process is limited, but there have been several studies on various aspects of sausage drying. TOWNSEND & DAVIS (1972) found that sausages placed in a horizontal position had lower yields than sausages hung in the normal, vertical position. LU & TOWNSEND (1973) studied the feasibility of adding freeze-dried meat to speed the drying of a fermented sausage and observed a shortening of the drying time. PALUMBO et al. (1976a) investigated the influence of variables such as fat content, meat type, cure type, meat particle size, casing diameter, pH, acidity, and fermentation method on sausage yield and found that only fat content and casing diameter greatly affected pepperoni yield. Additional work from our laboratory determined that sausage drying is a diffusional process (PALUMBO et al. 1977) and that sausage yield could be predicted by a newly developed rate equation.

Freezing is employed as a ready means of protecting meat from microbiological and chemical deterioration from time of production to time of consumption. According to recent figures (Livestock Market News, Weekly Summary and Statistics, 1980), almost 20% of both the pork and beef produced in the United States are held for varying time periods in frozen storage. There is only a very limited market for frozen red meats at the retail level. Frozen meat, especially pork, generally can be used only for processed products.

This study is a companion to the work of MILLER et al. (1980) on the effects of frozen storage on functionality of meat for "frankfurter" processing. They found that water holding capacity (WHC), total extractable protein, and emulsifying capacity of the thawed meats decreased significantly with length of time in frozen storage. In addition, flavor and texture of "frankfurters" processed from these meats decreased significantly with increasing time of frozen storage. With such a large percentage of red meats frozen, it is of interest and importance to extend the study of MILLER et al. (1980) to other processed meat products. The purpose of this study is to evaluate the influence of freezing the raw meats on sausage fermentation and drying kinetics.

Materials and Methods

Meat

Boneless cow chuck (from ungraded mature dairy cows), boneless pork shoulder, and pork backfat (both from U.S. #1 young swine) were obtained from local commercial slaughterhouses. The meats and fat were processed in our laboratory. First they were ground separately through a 1.91 cm plate on a Butcher Boy B52 grinder, then aliquots of each were ground twice through a 0.31 cm plate and analyzed for proximate composition (AOAC, 1975). From the analyses of these aliquots, beef (3 kg), pork (3 kg) and pork backfat (0.6 kg) were packaged together in polyethylene (Cry-O-Vac) bags. This proportion of beef, pork, and pork backfat gave a dry sausage mix with 18% fat content at the start of drying. One bag of the pork-beef-pork backfat was used to prepare pepperoni for each freezing time interval studied. The meats used in this study are the same as those by MILLER et al. (1980) for their experiment 1.

All samples to be frozen were placed in a freezer at −31.7 °C for 24 hours and then transferred to a freezer at −17.8 °C for the allotted storage time. Dry sausage was made at scheduled intervals for testing. For each test a bag of the beef-pork-pork backfat was removed from frozen storage and thawed in a coldroom at 0.5 °C for ca 48 hours, which minimized drip; any drip found in the bag was poured over the meat-fat mixture and was reabsorbed by the still slightly frozed meat. The zero time pepperoni were prepared from meat which had not been frozen.

Spices

The spices used for this study were purchased fresh from a local spice supplier at the start of the study and the same batch was used throughout. The proportions added to the meats were those described by PALUMBO et al. (1976b) except that the pimento was omitted. The spices were refrigerated (5 °C) during the 90-week study. For sensory evaluation at the end of the study, the pepperoni were prepared with fresh spices obtained from a local market.

Preparation of Dry Sausage

Standard pork-beef (1:1) pepperoni were formulated, fermented for 24 hours at 35 °C, and dried at 12 °C and 65% relative humidity for 6 weeks as described previously (PALUMBO et al. 1976a, b; 1977). Fermentation was accomplished by the addition of Lactacel MC starter culture (Microlife, Inc., Sarasota, Fla.), according to the directions of the manufacturer. The cure was 150 ppm sodium nitrite (150 mg $NaNO_2$/kg meat mixture) and the salt level was 3½% as required by FSQS for the destruction of trichinae by curing (Meat and Poultry Inspection Regulations, 1979).

pH and Titratable Acidity

The pH and titratable acidity were determined on aqueous extracts of the pepperoni mixture before fermentation and after fermentation, just prior to drying (PALUMBO et al. 1976b).

Yield and Kinetics of Drying

Dry sausage % yield was calculated as described previously (PALUMBO et al. 1977). Predicted yield, rate constants, and other

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kinetic parameters were determined at weekly intervals during drying on triplicate sausage samples by the previously described rate equation (PALUMBO et al. 1977):

$$\frac{dx}{dt} = -k \left[\frac{x-c}{x}\right]^2$$

where x is the % yield at any time (t), k is the rate constant, and c is ultimate % yield at $t = \infty$. Yield and rate constant data were analyzed by analysis of variance and for linear regression (STEELE & TORRIE, 1960).

Nitrite and Nitrosamines

The nitrite content of the pepperoni after drying was determined by the method of FIDDLER (1977). Nitrosamines were determined on the pepperoni after drying by a modification of the procedure described by FINE et al. (1975a). A 25 g ground sample, to which was added 1 ml CH_2Cl_2 solution containing 0.25 µg/ml N-nitrosomethylethylamine and 0.25 µg/ml N-nitrosohexamethyleneimine as internal standards, was placed in a 500 ml, 2-neck distillation flask equipped with a thermometer and a stopcock connecting tube. Fifty ml of mineral oil and 4 ml of 0.1 N NaOH were added, and the sample was distilled under vacuum (0.5 mm Hg) until a temperature of 140 °C was achieved. The distillate was collected in a glass trap immersed in liquid nitrogen and then quantitatively transferred to a 250-ml separatory funnel with 5 ml H_2O then 5 ml CH_2Cl_2. After the addition of 4 ml of 0.1 N HCl, the distillate was extracted three times with an equal volume of CH_2Cl_2. The combined extracts were dried by passage through anhydrous sodium sulfate and concentrated to 0.1 ml in a Kuderna-Danish apparatus. The concentration of volatile nitrosamines was determined quantitatively by GLC-thermal energy analyzer (TEA) under conditions similar to those described by FINE et al. (1975b) except that the cold trap for the TEA was immersed in a liquid nitrogen-ethanol slurry at −117 °C.

Sensory Panel

Informal taste testing at the start of the study indicated that pepperoni prepared from meat that had been frozen for increasingly longer periods had less flavor than freshly prepared pepperoni. To study flavor change in detail, additional pepperoni were prepared at the last storage time (90 weeks) with both old spice (OS, original spice, held 90 weeks at 5 °C) and new spice (NS, purchased just prior to use) and old meat (OM, meat held 90 weeks at −17.8 °C) and new meat (NM, purchased just prior to use — never frozen) for sensory evaluation. The pepperoni samples prepared for testing were: OS with OM, OS with NM, NS with OM, and NS with NM. Sensory evaluations were made by a panel of 16 laboratory members experienced in tasting cured meat products. For sensory evaluation, samples of finished pepperoni were sliced and whole slices were presented to the panelists under green light. NS with NM was designated as the standard and assigned a value of 10. Panelists were asked to compare each sample for pepperoni flavor to the standard and assign it a numerical value on a scale of 0 to 10. A hidden control was included in the comparisons. The sensory panel data were analyzed by analysis of variance and Duncan's new multiple range test (STEELE & TORRIE, 1960).

Tab. 1: Effects of time in frozen storage (at −17.8 °C) on pH and titratable acidity (TA) of sausage before fermentation and after fermentation just prior to drying

Weeks in frozen storage	pH[a]		% TA[a]	
	Before fermentation	After fermentation	Before fermantation	After fermentation
0	5.82	4.50	0.30	0.57
1	5.88	4.58	0.28	0.58
7	5.70	4.50	0.31	0.55
13	5.80	4.50	0.25	0.47
19	5.80	4.50	0.28	0.55
25	5.80	4.40	0.25	0.55
74	6.00	4.70	0.20	0.43
90	6.00	4.70	0.22	0.47

a) Single determinations

Results and Discussion

Fermentation

The effects of time in frozen storage (−17.8 °C) on pH and titratable acidity (TA) of pepperoni mix before fermentation and of the fermented pepperoni just prior to drying are given in Tab. 1. The pH of the meat before fermentation increased slightly during the course of frozen storage because of enzymatic and chemical breakdown of proteins with the release of amines and ammonia (PATTERSON & EDWARDS, 1975). This breakdown is also reflected in a decrease in the TA. However, the pH drop and TA increase caused by fermentation were essentially the same throughout the study. These comparable fermentation changes indicate a constancy of fermentative activity for the different batches of starter culture used during the study. Further, no compounds that interfere with fermentation appear to be generated or released from the meat during frozen storage.

Yield and Drying Kinetics

MILLER et al. (1980) reported that the WHC, total extractable protein, and emulsifying capacity of the raw meats had decreased significantly during frozen storage. Thus, it was anticipated that many of the parameters reflecting pepperoni drying should change with time. The effects of time in frozen storage on pepperoni yield at 6 weeks (the normal drying period) are given in Tab. 2; statistical evaluation of these data by regression analysis indicated no change ($p > 0.05$) in the % yield with length of time in frozen storage. Since final moisture content (yield at 6 weeks) is a function of the relative humidity of the dry room (PALUMBO et al. 1977), it was thought that early (3 weeks) yield might be affected by length of frozen storage; however, 3-weeks yields also were not affected ($p > 0.05$) by length of frozen storage (Tab. 2). MILLER et al. (1980) found that, despite the fact that the WHC of the meats decreased with increased frozen storage time, frankfurter yield also was not affected. A possible explanation may be that the salt added during formulation (3⅓% for pepperoni and 2½% for "frankfurters") can compensate for the decrease in WHC of the starting meats. In addition, though the decrease in WHC of the meats is statistically significant, the WHC even at the end of extensive frozen storage may still be high enough as not to affect final moisture content of the products.

Various kinetic drying parameters were calculated from the rate equation. Drying rate constants were not affected ($p > 0.05$) by frozen storage of the dry sausage ingredients (Tab. 3). The rate equation (PALUMBO et al. 1977) and % yield data for the first three weeks of drying can also be used to generate a prediction of the % yield at 6 weeks. The predicted % yields at 6 weeks (Tab. 2) are slightly higher than the actual 6 week % yields as observed previously (PALUMBO et al. 1977); they also showed no trend ($p > 0.05$) with the length of time the meat was frozen. Mean square data (not presented), which are a measure of the goodness of fit of actual data to the theoretical rate equation, again showed no trend ($p > 0.05$) with time. Pepperoni processing is not as sensitive to deterioration in the thawed pork and beef as is "frankfurter" processing (MILLER et al. 1980).

Nitrosamines and Residual Nitrite

The residual nitrite in pepperoni after drying was extremely low

Tab. 2: Effects of length of time of meat in frozen storage (at −17.8 °C) on dry sausage yield at 3 and 6 weeks of drying

Weeks in frozen storage	Mean[a] % yield at		
	3 Weeks of drying	6 Weeks of drying	
		actual	predicted[b]
0	59.0	52.7	54.0
1	54.9	49.5	50.3
7	57.9	51.7	52.1
13	58.7	52.1	53.0
19	56.2	50.3	51.3
25	54.2	50.3	51.3
74	60.3	52.8	53.4
90	58.5	51.7	53.0

a) Average of three sausages

b) Calculated from rate equation and % yield for the first three weeks of drying

Tab. 3: Efects of time of meat in frozen storage (at −17.8 °C) on drying rate constants (calculated from weekly % yield data and the rate equation)

Weeks in frozen storage	Mean[a]) drying rate constant
0	25.8
1	25.3
7	19.0
13	22.1
19	24.3
25	25.3
74	20.8
90	21.7

a) Average of three sausages

Tab. 4: Effects of length of time of meat in frozen storage (at −17.8 °C) on nitrosamines and residual nitrite in pepperoni after fermentation and 6 weeks of drying

Weeks in frozen storage	Residual nitrite, ppm	Nitrosamines ppb
0	4	none
1	0	none
7	0	none
13	1	none
19	0	none
25	1	none
74	0	1 dimethylnitrosamine/ 1 nitrosopyrrolidine[a])
90	2	0,5 dimethylnitrosamine[a])

a) By TEA, below the level confirmable by mass spectrometry

Tab. 5: Effects of age of ingredients[a]) on pepperoni flavor

Peperoni ingredients		Mean taste score
Meat	Spice	
new	new	8.55[bac])
new	old	6.05 b)
old	old	4.93 b)
old	new	4.56 b)

a) New: meat and spice were purchased just prior to the preparation of the pepperoni, meat was never frozen
Old: meat, frozen for 90 wk at −17.8 °C
Old: spice, refrigerated for 90 wk at 5 °C.

b) Hidden control: all samples were compared to a standard pepperoni prepared from new meat and new spice and assigned a value of 10

c) Means within the same column having one of the same letter are not significantly different at the 95% confidence level.

(Tab. 4) and was not related to the length of time the meats were in frozen storage. Nitrosamines were not found in confirmable quantities in any of the samples tested, even in those pepperoni prepared from meat frozen 74 and 90 weeks. The potential does exist for nitrosamine formation in pepperoni prepared from meat frozen for extended periods because of the development of amines. PATTERSON & EDWARDS (1975) showed that the amine content of the pork increased with extended frozen storage. MIRVISH (1970) reported that nitrosation is a function of increased amine level and the reaction increases as the pH is lowered to 3.5. The lengthy drying period (42 days) at pH values well below 5.0 provide ample opportunity for nitrosamine formation. In addition, the lactic microflora, known to contribute to nitrosamine formation (COLLINS-THOMPSON et al. 1972; HAWKSWORTH & HILL, 1971a, b), remains viable during the drying period (PALUMBO et al., 1976b). The absence of nitrosamines in pepperoni parallels an earlier finding that there were no nitrosamines in Lebanon bologna, a fermented all beef sausage product (PALUMBO et al. 1974). Nitrosamines were not found in Thuringer (DETHMERS et al. 1975) or European dry sausage (KOTTER et al. 1976).

Taste Panel

The taste panelists detected a significant decrease in flavor of pepperoni made from old ingredients whether these were spices (held 90 weeks at 5 °C) or meat (held 90 weeks at −17.8 °C) (Tab. 5).

Because of the manner in which the sensory panel tests were made, it could not be determined when during frozen storage the meat would yield pepperoni with lowered flavor score. However, MILLER et al. (1980) observed that "frankfurters" made from the same frozen meat and evaluated by the triangle test were differentiated from the control ("frankfurters" made from non-frozen meat) first on textural loss and only after extended frozen storage on flavor loss. The sensory panel scores (Tab. 5) indicated that use of even fresh spices with old meat could not yield an acceptable quality product, since spices themselves deteriorate upon extended refrigerated storage (TANDLER et al. 1977). Thus both fresh meat and fresh spices are needed to yield quality pepperoni.

In conclusion, we observed no change in sausage fermentation and drying kinetics in pepperoni prepared from frozen meat. However, extended frozen storage of the meat and/or refrigerated storage of the spices did yield pepperoni with lowered flavor scores. The results of this study should be applicable to other dry fermented sausages. Since storage of both meats and spices adversely affected pepperoni flavor, it is suggested that only fresh ingredients be used to prepare this type of product.

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Notes

Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

Precautions should be exercised in the handling of nitrosamines since they are potential carcinogens.

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