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Studies on the Color of Meat and Meat Products

II. Effects of Polyphosphates on the Color of Cooked Sausage

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As briefly reviewed in the previous paper[1], according to the results of works published previously[2~8], the detailed action of phosphates on the color of meat products is still far from being explicitly elucidated.

It was found in our previous work that the addition of acidic orthophosphates gave favorable effects on the color of cooked sausage, but resulted in considable decrease in water holding capacity of meat, whereas the supply of basic orthophosphates developed a tendency to exert undesirable influences on color and encouraging influences on water retention by meat.

The present study has been made to investigate the behavior of polyphosphates towards the formation and the protection of color of cooked cured meat.

METHODS

In the present experiment, color formation value (CFV), color retention value (CRV), pH value and TBA absorbance were determined in the same ways as described in the previous paper, but determinations of water holding capacity of meat, reducing value, nitrite and volatile basic nitrogen were made by modified methods given below.

Preparation of cooked sausage:

Beef chuck at five days after slaughter was trimmed to remove fat and connective tissue as far as possible, and the resulting lean meat was then ground three times with a meat grinder. The ground meat was mixed thoroughly and was divided into five lots.

Sausage samples were prepared in the same manner as related in the previous paper, namely, each lot was treated with each of the mixture of curing ingredients given in Table 1, stuffed into Krehalon (vinylidene chloride-vinyl chloride copolymer) casing, cured for 72 hours at 4°C, cooked at 75°C for an hour, and then cooled.

Determination of water holding capacity of meat:

The total water content of ground fresh meat sample was determined in the usual way, and the amount of cooking loss was determined in the same manner as described in the previous paper except for the modifications in the following two points.

First, cooking loss was separated at 7,000 rpm for 10 minutes instead of separating at 2,500 rpm for 10 minutes. Second, since usually a certain amount of many sorts of inorganic and organic compounds are contained in cooking loss, the water content of cooking loss was determined in the usual way by drying at 100 °C, and the water holding capacity ot meat was calculated by the following formula:

$$\text{Water holding capacity of meat} = \frac{\%\ \text{total water} - \%\ \text{water of cooking loss}}{\%\ \text{total water}} \times 100$$

By repeating experiments on centrifugal

Table 1. Curing ingredients used for the preparation of cooked sausage

Sample number	Curing ingredients				
	NaCl	$NaNO_2$	Polyphosphate[a]		Neofurasukin[b]
Control	2.5 %	0.02 %	—	—%	0.025 %
1	2.5	0.02	$Na_2H_2P_2O_7$	0.5	0.025
2	2.5	0.02	$Na_4P_2O_7$	0.5	0.025
3	2.5	0.02	$Na_5P_3O_{10}$	0.5	0.025
4	2.5	0 02	$Na_6P_6O_{18}$	0.5	0.025

(a) Each amount of the polyphosphate added was calculated on the basis of anhydride.
(b) Preservative permitted in Japan. Neofurasukin is a mixture of nitrofurazone and nitrofurylacryl amide.

separation of cooking loss from cooked ground meat at 4,000, 5,000, 6,000, 7,000, 8,000, 9,000 10,000 rpm for 10 minutes, respectively, it was found that most successful results were obtained when samples were centrifuged at 7,000 rpm for 10 minutes, because the variation in values for cooking loss separated from the very same samples was the slightest, viz., the coefficient of variation of the findings for cooking loss obtained from the same samples showed the lowest value, and even a trifling difference in cooking loss between two different samples could be observed most explicitly.

Besides, through determining the water content of cooking loss, it was possible to get indisputably more accurate figures for water holding capacity of meat than before.

Determination of reducing value :

As a result of further experiments on the determination of reducing value of meat and meat products, the following modification of the method of Kajita[9] was found to be most successful.

Two grams of a meat sample were homogenized with 5 ml of distilled water for 3 minutes, cooling with ice water. The homogenate was transferred into a 20 ml graduated test tube with 10 ml of M/15 phosphate buffer of pH 7 and with distilled water. After diluted to the 20 ml mark with distilled water, 2 ml of M/50 $K_3Fe(CN)_6$ solution were added, the test tube being stopped with a well-fitted glass stopper, mixed thoroughly, and the mixture was allowed to stand for 20 minutes at 0 °C. Afterwards the mixture was removed into a 100 ml beaker with 8 ml of distilled water, 10 ml of 10% trichloracetic acid solution was added, mixed thoroughly, and allowed to stand for 10 minutes at room temperature, then filtered through a filter paper. The optical absorption of the filtrate was measured at 420 mμ using a 1 cm cell, with a mixture, which underwent the same operation as before except that 4 ml distilled water were added instead of 2 g of meat sample and 2 ml of M/50 $K_3Fe(CN)_6$ solution, as a blank.

The mg % of cysteine was calculated by the following formula, and the finding for mg % of cysteine was expressed as RV (reducing value).

$$\text{RV (mg \% of cysteine)} = 242.32 - 224.37\,A$$

A = Absorbance of sample observed.

Determination of nitrite :

Since there are several methods for the determination of nitrite[10-14], considerable efforts have been exerted to find out the most satisfactory method for determining nitrite present in meat and meat products. From the results given by repeated examinations, the following procedure, a modified method of Shinn[11], may confidently be recommended.

Two grams of a meat sample were homogenized with 5 ml of distilled water in a Waring blender for 3 minutes. The homogenate was transferred into a 150 ml graduated 300 ml beaker with enough distilled water heated to 80 °C to fill to the 150 ml mark. The beaker was placed on a boiling water bath and heated for an hour, the content being stirred occasionally with a glass rod. The evaporated water during the heating process was supplied occasionally by adding distilled water to the 150 ml mark throughout the heating operation. Two ml of saturated $HgCl_2$ solution were added, mixed, and cooled with running tap water to room temperature, then transferred into a 200 ml volumetric flask, diluted to the mark with distilled water, mixed thoroughly, filtered through a filter paper.

To 8 ml of the filtrate 2 ml of reagent (a mixture of 1.0 ml of 0.2% sulfanilamide aqueous solution, 0.2 ml of 6 NHCl solution, 0.2 ml of 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride aqueous solution, and 0.6 ml of distilled water) were added, mixed, and kept at 30 °C for 30 minutes, cooled with running tap water for 10 minutes, transferred into a 1 cm cell and the absorbance was determined at 535 mμ with a mixture of 8 ml distilled water and 2 ml reagent as a blank. The content of nitrite was expressed as ppm of nitrite group (NO_2^-).

Determination of volatile basic nitrogen:

In the present experiment, the content of volatile basic nitrogen was determined by a modification of the micro-diffusion method of Conway[15].

RESULTS AND DISCUSSION

Color formation value (CFV) and color retention value (CRV):

According to the results given in Table 2, the addition of disodium pyrophosphate ($Na_2H_2P_2O_7$) decreased the pH value to some extent and produced most favorable effects on the color of cooked sausage, i. e., each value of the sample supplied by disodium pyrophosphate for both CFV and CRV always showed the highest figure among the five kinds of cooked sausage samples. When treated with sodium hexametaphosphate ($Na_6P_6O_{18}$), no practically significant change in pH value was effected and some favorable effects were brought about on the color of cooked sausage. Most considerable increase of pH value and most undesirable effects on color were observed in the sam ple treated with tetrasodium pyrophosphate ($Na_4P_2O_7$), and the addition of sodium tripolyphosphate ($Na_5P_3O_{10}$) exhibited to develop the same tendency as displayed by the supply of tetrasodium pyrophosphate, though the influence of sodium tripolyphosphate was expressly less effective than that of tetrasodium pyrophosphate.

These results may be taken to indicate, as previously related in cases of orthophosphates, that the decrease in pH value by supplying acidic polyphosphate, viz. disodium pyrophosphate, would manifest desirable effects on color formation and protection from discoloration by light, whereas the increase in pH by supplying basic polyphosphate, i. e. tetrasodium pyrophosphate and sodium tripolyphospate, would produce undesirable effects on color of cooked sausage.

Table 2. Effects of polyphosphates on pH, CFV, CRV and water holding capacity

Sample number	Polyphosphate added, 0.5%	Just after preparation					After storage for 4 weeks at 4°C					Water holding capacity
		pH	Before exposing to light		After exposing to light		pH	Before exposing to light		After exposing to light		
			Absorbance	CFV	Absorbance	CRV		Absorbance	CFV	Absorbance	CRV	
Control	None	6.05	1.028	100	0.468	46	6.05	0.935	91	0.314	31	55.48
1	$Na_2H_2P_2O_7$	5.95	1.076	105	0.518	50	5.95	0.978	95	0.382	37	53.23
2	$Na_4P_2O_7$	6.35	0.941	92	0.341	33	6.36	0.885	86	0.257	25	72.98
3	$Na_5P_3O_{10}$	6.25	0.984	96	0.371	36	6.24	0.921	90	0.295	29	69.70
4	$Na_6P_6O_{18}$	6.04	1.046	102	0.487	47	6.03	0.979	95	0.358	35	56.91

It seems to be conceivable, however, that changes in some factors other than pH resulted from the supply of polyphosphates might exert some particular influences upon the color of cooked sausage.

As shown in Table 2, no significant changes occurred in pH values during the storage period of 4 weeks at 4 °C, but a considerable decrease took place in values for CFV and CRV during the storage.

Water holding capacity of meat:

In almost the same manner as manifested in the previous experiment on orthophosphates, the water holding capacity of meat was most remarkably improved by the addition of most basic polyphosphate, i. e. tetrasodium pyrophosphate, and the effect of sodium tripolyphosphate on water holding capacity ranked next.

The addition of disodium pyrophosphate, an acidic polyphosphate, resulted in a slight decrease in pH value and a discouraging decline in the water holding capacity of meat, while the supply of sodium hexametaphosphate brought about an insignificant change in pH value and a just faint increase in the water holding capacity of meat.

According to the theories on the mechanism of the action of alkaline phosphates, increases in pH value, ionic strength and solubility of fibrous myofibrillar proteins caused by the addition of phosphates, abilities of phosphates to remove bivalent cations, such as calcium and magnesium, from peptide chains by complexing with those cations and to split actomyosin into its components myosin and actin, are considered to be most conceivably contributable to the favorable effects of phosphates on the water holding capacity of meat.

Since the main purpose of the present experiment is to investigate the effects of polyphosphates on the color of cooked sausage, nothing further should be here concerned with the problem of water holding capacity of meat.

Nevertheless, as pointed out in the previous paper, since the supply of polyphosphates produces quite an opposite effect on color to those on the water holding capacity of meat, in cases of orthophosphates as well, it should be forcibly emphasized here again that a prudent attitude must be taken when phosphates are put to practical use.

Reducing value (RV) and NO_2^-:

The supply of disodium pyrophosphate, the most acidic polyphosphate used for the present experiment, resulted in most considerable increase of RV and most remarkable decrease of NO_2^- content, whereas the addition of tetrasodium pyrophosphate, the most basic polyphosphate employed in the present experiment, resulted in most marked decrease of RV and most striking increase of NO_2^- content, as compared with the findings for control.

Since the high reducing power and the low pH value of meat promote the decomposition of nitrite into nitric oxide, and in consequence yields favorable results on the formation and retention of cooked cured meat color, it would be conceivably reasonable that the content of NO_2^- in cooked sausage may increase with decreasing reducing power and increasing pH value of cooked sausage, and that the higher remaining content of NO_2^- denotes the lesser formation of nitric oxide heme pigment and accordingly exerts infallibly discouraging influences on the color of cooked sausage.

But the reasons why the RV of the cooked sausage has remarkably increased by the addition of disodium pyrophosphate and why the RV of the cooked sausage significantly decreased by the treatment with tetrasodium pyrophosphate still remain unknown.

From the findings for volatile basic nitrogen and TBA absorbance given in Table 3, it would be conceivably suggested that faintly slight changes in protein and no significant changes in fat underwent during the storage, but consistent and evident decreases in the figures for NO_2^- content were observed during the storage.

As to the influence of polyphosphates on changes in the figures for RV during storage, many further experiments should be required before a definite conclusion can be safely drawn.

Table 3. Effects of polyphosphates on reducing value and NO_2^- content

Sample number	Polyphosphate added, 0.5%	Just after preparation				After storage for 4 weeks at 4 ℃			
		Reducing value	NO_2^-	Volatile basic N	TBA absorbance	Reducing value	NO_2^-	Volatile basic N	TBA absorbance
		Cysteine mg%	ppm	mg%		Cysteine mg%	ppm	mg%	
Control	None	64.2	46.0	18.84	0.274	45.0	30.8	21.10	0.188
1	$Na_2H_2P_2O_7$	77.8	36.8	19.92	0.268	50.2	22.8	21.97	0.184
2	$Na_4P_2O_7$	35.1	85.4	22.01	0.253	38.7	58.7	26.35	0.206
3	$Na_5P_3O_{10}$	41.4	73.4	19.91	0.258	42.9	46.2	24.17	0.222
4	$Na_6P_6O_{18}$	67.8	44.5	20.78	0.270	50.5	28.1	24.06	0.209

SUMMARY

A study on the effects of four kinds of polyphosphates ——disodium pyrophosphate ($Na_2H_2P_2O_7$), tetrasodium pyrophosphate ($Na_4P_2O_7$), sodium tripolyphosphate ($Na_5P_3O_{10}$), and sodium hexametaphosphate ($Na_6P_6O_{18}$) —— on the color of cooked sausage were performed in the present experiment.

The addition of disodium pyrophosphate resulted in a significant decrease in pH value and a remarkable increase in reducing power of meat, and accordingly promoted the decomposition of nitrite into nitric oxide, thus brought about the most favorable effects on color formation and protection from fading by light of all the cooked sausage samples employed in the present experiment.

Although no sinificant shift in pH value was effected, a slight increase in reducing power of meat and some encouraging effects on the color of cooked cured meat were manifested by the supply of sodium hexametaphosphate.

As a markedly considerable increase in pH value and a definite decrease in reducing power of meat resulted from the addition of tetrasodium pyrophosphate, a fairly large amount of nitrite was allowed to remain unchanged, and this deservedly brought about the most undesirable effects on producing and protecting cooked cured meat color of all the sausage samples used for the present experiment.

Quite similar tendencies, as displayed in the case of tetrasodium pyrophosphate, were observed when the meat sample was treated with sodium triphoshate, but every effect on pH value, nitrite content, reducing power of meat, development and retention of cooked cured meat color was consistently and explicitly less intensive than that achieved in the case of tetrasodium pyrophosphate.

Nevertheless, since the addition of polyphosphates which manifestly increases the water holding capacity of meat has always produced an indisputably discouraging effects on color of cooked sausage, and vice versa, some efficacious devices, which not only produce favorable effects on water holding capacity of meat but also promote explicitly desirable effects on color of cooked cured meat, seem to be indispensable when polyphosphates are put to practical use in processing meat products.

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SUMMARY

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Table Effects of polyphosphates on pH, CFV (color formation value), reducing value, NO_2^- content and water holding capacity of cooked sausage just after preparation.

Sample No.	Polyphosphate added, 0.5%	pH	CFV	Reducing value	NO_2^-	Water holding capacity
				Cysteine mg%	ppm	
Control	None	6.05	100	64.2	46.0	55.48
1	$Na_2H_2P_2O_7$	5.95	105	77.8	36.8	53.23
2	$Na_4P_2O_7$	6.35	92	35.1	85.4	72.98
3	$Na_5P_3O_{10}$	6.25	96	41.4	73.4	69.70
4	$Na_6P_6O_{18}$	6.04	102	67.8	44.5	56.91