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Effect of Inorganic Salts on the Strength of Muscle Fibers

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To study the effect of inorganic salts on the strength of muscle fibers, tensile strength was measured about the muscle fibers prepared from the chicken pectoralis major muscles after incubation with various kinds of salt solutions. The tensile strength of muscle fibers changed little between pH 5.0 and 8.0. Although monovalent cations and most of anions had a relatively small effect, divalent cations and phosphates decreased greatly the tensile strength of muscle fibers. The decreasing action of divalent cations and phosphates on the shear force value of muscles was also noted in the experiment of the rehydration of the lyophilized muscles in these salt solutions.

In previous work it was shown that the tensile strength of muscle fibers decreased greatly during postmortem aging and also in the presence of small amounts of Ca ions (Nakamura, 1972). From these results it was suggested that the cause of the changes in the tensile strength of muscle fibers might be the released Ca ions from muscles during postmortem aging. The action of Ca ions on the muscle fibers, however, has not been clarified yet.

In this work, in order to obtain more information about the action of Ca ions on muscle fibers, the effect of various inorganic salts on the tensile strength of muscle fibers was studied. The effect of the inorganic salts on the tenderness of aged meat was also studied.

EXPERIMENTAL SECTION

Pectoralis major muscles were obtained from 12- to 14-month-old chickens (White Leghorn, female). All birds were killed by cutting the jugular vein and carotid arteries, were skinned without scalding, and were eviscerated. The carcass was placed in a plastic bag and aged in drained crushed ice. Muscle fiber bundles were prepared at 1–2 hr after slaughter and kept in 50% glycerol as described previously (Nakamura, 1972). They were dipped in 50% glycerol to which various amounts of inorganic salts were added, were kept overnight at 0°, and their tensile strength was measured. Each muscle bundle was 2–3 cm in length and contained about 25 single fibers.

Tensile strength measurements were made on about 8–10 muscle fiber bundles by the use of a strain gauge attached to an automatic recorder (Nakamura, 1972).

Shear force value was measured about the muscles aged for 24 hr at 0°. To study the effect of inorganic salts, muscles (approximately 5–7 cm wide, 15–18 cm long, and 1–1.5 cm thick) were lyophilized and rehydrated in 5 m*M* solution of various inorganic salts for 2 days at 0°. A small amount of thymol was added to each solution to suppress the bacterial growth during rehydration. Shear force value was measured by a Warner–Bratzler type apparatus after cooking; muscle samples were clamped in a special mold designed according to de Fremery and Pool (1960) and cooked to an internal temperature of 82–85° (about 1 hr in boiling water). Strips 0.25 cm^2 in cross-section were cut and 8–12 determinations were made on each sample. To obviate the effect of bird-to-bird variation, comparisons were made between left and right halves of one bird; one half was dipped in 5 m*M* KCl solution as control sample and the other half was dipped in various kinds of salt solutions with the same concentration.

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RESULTS AND DISCUSSIONS

The tensile strength of muscle fibers did not change with the addition of KCl or NaCl until the concentration of 20 m*M* was reached (Table I). As this concentration is much higher than that of the effective concentration of $CaCl_2$ described in the previous work (Nakamura, 1972), 3 m*M*, the effect of $CaCl_2$ on the muscle fibers seems to be rather specific.

To compare the effect of $CaCl_2$ with that of other inorganic salts, experiments were made about various kinds of inorganic salts. In this case, there is a possibility that the effect of inorganic salts is that of the change in pH caused by their presence. As shown in Figure 1, the tensile strength of muscle fibers was affected greatly by the pH of the solution; it decreased greatly below pH 5.2 and above pH 8.1. Most pH of the glycerol solution with various amounts of inorganic salts tested, however, was between 5 and 8. When the pH of the glycerol solution tested was above 8 or below 5 (in the case of either 10 m*M* K_2HPO_4 or 10 m*M* KH_2PO_4 in Table II), comparisons were not made with other experimental results. This means that the change in pH caused by the addition of

Table I. Effect of the Concentration of KCl or NaCl on the Tensile Strength of Muscle Fibers (g/25 Single Fibers) (Mean ±SD)

	KCl				NaCl	
None	5 m*M*	10 m*M*	20 m*M*	40 m*M*	20 m*M*	40 m*M*
2.2 ± 0.3	2.2 ± 0.3	2.3 ± 0.3	2.2 ± 0.3	1.6 ± 0.3	2.2 ± 0.2	1.8 ± 0.5

Table II. Effect of Anions on the Tensile Strength of Muscle Fibers (g/25 Single Fibers) (Mean ±SD)

	Group I[a]						Group II[b]			
	KI	KNO_3	KCl	CH_3-COOK	K_2SO_4	K-Tartrate	K_2HPO_4	KH_2PO_4	Na-Tripolyphosphate	Na-Hexametaphosphate
5 m*M*	2.2 ± 0.3	2.0 ± 0.2	2.2 ± 0.3	1.9 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	1.2 ± 0.3[e]	1.6 ± 0.3[e]	0.6 ± 0.2[e]	0.5 ± 0.2[e]
10 m*M*	2.2 ± 0.3	2.0 ± 0.2	2.2 ± 0.3	1.9 ± 0.3	1.6 ± 0.3[e]	1.3 ± 0.3[e]	(1.0 ± 0.3)[c,e]	(1.2 ± 0.2)[d,e]	0.5 ± 0.2[e]	0.5 ± 0.2[e]

[a] The order of Group I is that of the Hofmeister series of anions. [b] Group II contains various kinds of phosphate compounds. [c] pH of this solution was 8.3. [d] pH of this solution was 4.8. [e] Significantly different from KCl-treated muscle fibers (<0.05).

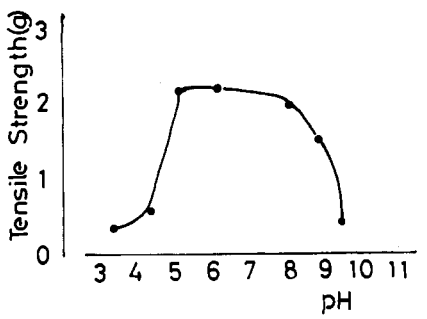

Figure 1. Effect of pH on the tensile strength of muscle fibers (g/25 single fibers)

inorganic salts does not affect the result of the tensile strength measurement in this study.

When the relation between inorganic salts and macromolecules is studied, the dehydrating action of inorganic salts which is represented as Hofmeister series is often considered. Almost all the anions of the Hofmeister series (Group I anions in Table II), however, had a relatively small effect on the tensile strength of muscle fibers except for sulfate and tartrate, which had a slightly decreasing effect (Table II). As the dehydrating action of Group I anions varies greatly (Young, 1963), this seems to show that the decrease in the tensile strength of muscle fibers is not due to the dehydrating action of inorganic salts. This was ascertained further by the experiment with various cations (Table III); all the monovalent cations with different dehydrating action did not affect the tensile strength of muscle fibers. On the other hand, phosphates (Group II anions in Table II) and divalent cations (Table III) had a large decreasing effect on the tensile strength of muscle fibers. Based on the above discussions, the action of these salts is considered not due to their dehydrating action.

The decreasing action of divalent cations and phosphates on the shear force value of aged muscles was also noted in the experiment of the rehydration of the lyophilized meat in these salt solutions; although the shear force value of the lyophilized–rehydrated meat in KCl solution was almost the same as that of untreated meat, that of the meat treated with divalent cations or phosphates was small (Table IV). To analyze the variance for KCl treatment and $CaCl_2$ or polyphosphate treatment statistically, an experiment was made about seven birds. The result (Table V) showed a highly significant effect of the $CaCl_2$ or polyphosphate treatment on the shear force value of meat. Experiments were made about intact muscles by dipping them in various kinds of inorganic salt solutions. However, any effect of treatment could not be found. Considering the experiment of lyophilized–rehydrated meat, this seems to depend on the difficulty in the penetration of inorganic salts into the muscles.

All these results seem to show that the divalent cations or phosphates had a special action on the muscle fibers. It was already suggested that both ATP and polyphosphates would dissociate actomyosin into actin and myosin (Bendall, 1954). This is considered to be the cause of both the low tensile strength of muscle fibers and the low shear force value of the muscles treated by polyphosphates. However, 5 m*M* ATP did not decrease the tensile strength of muscle fibers at all (Nakamura, 1972). This result seems to show that the other cause besides the dissociation of actomyosin must be present in case of polyphosphate treatment. No studies have been made about this problem. Further work should be made. Haga *et al.* (1966) reported that Ca ions weakened the myo-

Table III. Effect of Cations on the Tensile Strength of Muscle Fibers (g/25 Single Fibers) (Mean ±SD)

	KCl	NaCl	LiCl	$CaCl_2$	$MgCl_2$	$BaCl_2$
5 m*M*	2.2 ± 0.3	2.2 ± 0.3	2.1 ± 0.3	0.7 ± 0.3[a]	1.3 ± 0.2[a]	0.7 ± 0.2[a]
10 m*M*	2.2 ± 0.3	2.3 ± 0.3	2.2 ± 0.2	0.5 ± 0.3[a]	0.6 ± 0.2[a]	0.5 ± 0.2[a]

[a] Significantly different from KCl-treated muscle fibers (<0.05).

Table IV. Effect of Inorganic Salts on the Shear Force Value of Aged Meat (kg) (Mean of Three Birds ±SD)[a,b]

	Lyophilized–rehydrated meat						
Intact meat	KCl	$MgCl_2$	$CaCl_2$	$BaCl_2$	K_2HPO_4	Na-Hexametaphosphate	Na-Tripolyphosphate
1.5 ± 0.2	1.6 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	1.0 ± 0.2	0.8 ± 0.2

[a] Five-m*M* salt solution was used for the rehydration of lyophilized meat. [b] The difference of KCl-treated meat among every three birds was within the range of the standard deviation. So, the result was summarized in a single table.

Table V. Analysis of Variance for KCl Treatment and $CaCl_2$ or Tripolyphosphate Treatment of Lyophilized Meat

	df	KCl	$CaCl_2$ or tripoly-phos-phate	Mean pair difference	p
KCl–$CaCl_2$	6	1.6	1.1	0.5	<0.05
KCl–tripoly-phosphate	6	1.8	1.0	0.8	<0.01

fibril at the z lines, and Davey and Gilbert (1969) showed that EDTA suppressed the morphological changes in the myofibril during postmortem aging. As the amount of water-extractable Ca in meat increases during postmortem aging (Arnold *et al.*, 1956), this special effect of Ca ions is very interesting in the study of meat tenderization phenomenon. Studies are now in progress about the factors which affect the release of Ca ions from meat during postmortem aging.

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Pectinase Stabilization of Orange Juice Cloud

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The cloud of fresh orange juice was stabilized without heating by adding a commercial pectinase at 200 ppm. The level of stable cloud was proportional to pectinase concentration and to treatment temperature. Six other commercial pectinases were tested for stabilizing orange juice cloud. One pectinase accelerated clarification, but the other five stabilized with varying effectiveness. Pectin distribution analysis, pectinesterase assay of pectinase-treated juice, and pectin depolymerizing activity assays of the pectinases show the following. Pectinases degrade orange juice particulates and release bound pectinesterase into the juice. Pectinases stabilized orange juice cloud by depolymerizing pectic substances to soluble pectates instead of to insoluble pectates. And effectiveness of stabilization correlates with the ratio of depolymerizing activity on polygalacturonic acid to that on pectin. Selection of pectinases to stabilize natural orange juice cloud can be made on the basis of their depolymerizing activities.

Fresh orange juice contains finely divided particulates in suspension that give it a "cloudy" appearance. Analyses have shown this particulate material is composed almost exclusively of pectin, protein, and lipid (Baker and Bruemmer, 1969; Scott *et al.*, 1965). [Mizrahi and Berk (1970) found substantial quantities of hesperidin crystals in the juice cloud of Shamuti orange, but this characteristic appears to be peculiar to the Shamuti variety.] When this unstable colloidal system collapses, the juice clarifies. Once converted to an unattractive two-phase system of a flocculant sediment in a clear serum, the juice is no longer marketable. In addition, the cloud, which will not remain suspended, contains most of the characteristic orange flavor and color.

Heat is used commercially to stabilize orange juice against cloud loss. Because a temperature near 90° is required, processed orange juice sometimes acquires off-flavors from excessive heat (Bissett *et al.*, 1953; Kew and Veldhuis 1960, 1961). Heating stabilizes orange juice cloud by inactivating pectinesterase (PE), an enzyme that initiates a series of reactions leading to clarification. PE demethylates juice-soluble pectin, converting it to low-methoxyl pectin, which reacts with polyvalent cations to form insoluble pectates. Presumably, the precipitation of these pectates occludes the cloud particles and removes them from suspension (Dietz and Rouse, 1953). Until recently, juice-soluble pectin was presumed to form a colloidal matrix that supports the particulates and therefore was necessary for orange juice cloud stability (Rouse and Atkins, 1955).

We showed that soluble pectin was not necessary for cloud support, as a stable suspension of orange juice particulates could be made in water (Baker and Bruemmer, 1969). Because pectin is the source of the destabilizing low-methoxyl pectin, we proposed controlled pectin degradation as an alternative to heat denaturation of PE for stabilizing orange juice cloud. The proposal was supported by the stability of orange juice particulates in centrifugally prepared orange juice serum treated with a commercial pectinase (Baker and Bruemmer, 1969). As used throughout this paper, the term pectinase refers to commercial pectolytic enzyme preparations.

Demonstration that orange juice particulates formed a stable cloud in orange juice serum containing a commercial pectinase suggested that orange juice might be stabilized by adding a pectinase directly to whole juice. The present paper reports the first instance of stabilizing orange juice cloud by adding ppm levels of commercial pectinases to whole juice.

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