Compensation of high-pressure processing for the solubility of sodium-reduced chicken breast myosin with three anion types of potassium salts

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ABSTRACT The effect of high-pressure processing (200 MPa, 10 min) on the solubility of chicken breast myosin with 25% molar substitution of Na^+ by 3 anion types of potassium salts (KCl, K-lactate, and K-citrate) was investigated. The results showed that the lower hydrophobic group and reactive sulfhydryl group of non-pressurized myosin with the replacement of organic K-lactate or K-citrate possibly contributed to the aggregation of myosin molecules compared with the KCl group and thus decreased the solubility of both. In the presence of lactate or citrate, the high-pressure processing caused an increase in the surface hydrophobicity and reactive sulfhydryl group, indicating the unfolding of myosin molecule. Meanwhile, the increased hydration state and the decreased apparent viscosity suggested the disruption of protein–protein interactions and the strengthening of myosin–water interactions in pressurized myosin, ultimately resulting in increased solubility of the pressurized myosin with both organic potassium salts. The compensation of high-pressure processing is interesting for the efficient selection of the anion type in developing sodium-reduced industrial meat products.

Key words: chicken breast myosin, solubility, high-pressure processing, anion types of potassium salts, sodium-reduced

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INTRODUCTION

Solubility is a critical functional property of myosin for emulsification and gelation of myofibrillar proteins, which are responsible for the water/fat-binding and texture of meat products (Guo et al., 2015). Usually, a high concentration of sodium chloride (**NaCl**) of 2–3% (0.47–0.68 mol) is required to solubilize the proteins to achieve the desired functional properties (Cando et al., 2015). However, there is an urgent need for low-sodium meat products as excessive sodium salt consumption can induce hypertension and cardiovascular diseases (WHO, 2013). Therefore, the improvement of myosin solubility under sodium-reduced conditions is of great significance for the meat industry.

Efforts to reduce sodium in processed meat products have been mainly focused on the partial replacement of sodium with other cations (Horita et al., 2011), and much is known about the effects of cations on meat proteins (Zhang et al., 2017a). However, binding of the negatively charged ions is considered stronger than the positively charged ones in myofibrillar proteins (Hamm, 1961; Puolanne and Halonen, 2010), and the stability of proteins is dependent primarily on the anion (Von Hipple and Wong, 1964). Recently, anion-induced effects on stability, solubility, and aggregation of proteins were noted (Yoshizawa et al., 2016), and the "salt-in" effect of the cations could be interfered by the presence of highly hydrated anions (Wu et al., 2016). However, the role of anions on the solubilization of myofibrillar proteins has not yet been elucidated.

Various potassium-based salt substitutes have been used in the development of low-salt meat products because of their antihypertensive properties and a much higher recommended maximum intake level (Tahergorabi et al., 2012). Potassium chloride (**KCl**) is the most commonly used salt substitute (Zhang et al., 2016). The organic potassium lactate or citrate (**K-lactate** or **K-citrate**), which is

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usually used as antimicrobial agents or saltiness enhancers in meat products, has become an alternative (Fulladosa et al., 2009; Fellendorf et al., 2016; Sansawat et al., 2019). These potassium salts also play an important role in preventive nutrition (Demigne et al., 2004).

The emerging high-pressure processing (**HPP**) technology can better meet the consumers' demands for safe and wholesome foods with fewer additives (Huang et al., 2017). Several studies have shown that the solubility of meat proteins can be improved by HPP (Sikes et al., 2009; Xue et al., 2018). However, the solubilizing effect of HPP depends on the system characteristics, such as the ionic strength and the type of salt, leads to a great challenge in the search for the right settings (Fernández-Martín et al., 2002). Notably, the interactions between protein and solvent are critical in determining the response of proteins to pressure (Boonyaratanakornkit et al., 2002), and HPP can also change the specific binding sites for anions (Speroni et al., 2014) and for water (Grossi et al., 2016; He et al., 2019). A previous study confirmed that HPP improved the water retention of sodium-reduced chicken breast gels containing K-lactate or K-citrate (Zhou et al., 2018). Furthermore, a myosin-based perspective study would be potentially useful for meat industry practices. To the author's knowledge, there is no literature investigating the impact of HPP on myosin solubility with different anion types of salts.

This research work investigated the effect of HPP (200 MPa, 10 min) on the solubility of chicken breast myosin in sodium-reduced solutions with 3 anion types of potassium salts (KCl, K-lactate, and K-citrate), which would be useful in further understanding the effects of anion types on HPP-myosin solubility and in providing a new sodium-reduced strategy for meat products.

MATERIALS AND METHODS

Materials

Chicken breast meat was purchased from the Carrefour Group, a local supermarket (Hefei, China), and stored at 4°C (within 4 h) until the extraction of myosin. NaCl, KCl, and K-citrate ($K_3C_6H_6O_7 \cdot H_2O$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). K-lactate ($C_3H_5KO_3$) solution (60%) was purchased from Aladdin Biological Technology Co., Ltd. (Shanghai, China). All chemicals used were of analytical grade.

Myosin Extraction

Myosin was extracted from chicken breast as described by Yao et al. (2018). The final obtained solution of protein was diluted with 9 volumes of cold distilled water and settled overnight, and then the precipitate was collected by centrifugation at 10,000 × *g* for 6 min at 4°C. The protein composition and concentration of the precipitate were determined by SDS-PAGE with a gel imaging system (Tanon 2500, Tanon Science Equipment Co., Ltd., China) and the Biuret method (Gornall et al., 1949).

Preparation of Myosin Solutions and HPP Treatment

The precipitated myosin was diluted in 4 different desired buffers to achieve a final concentration of 25 mg/mL and final different salt compositions as described in Table 1. The pH of the 4 buffers was adjusted to 6.0 with dilute HCl or KOH solution (the addition level had no significant influence on the ionic strength) before the dilution of precipitated myosin to exclude the effects of pH changes. All myosin solutions were independently prepared in triplicate.

The myosin solution was vacuum-packed in polyethylene bag (approximately 100 mL/bag) before HPP. High-pressure processing was performed in a 0.6-L-capacity, 600-MPa high-pressure vessel (BaoTou KeFa High-Pressure Technology Co., China), and 200 MPa for 10 min was chosen according to the method of Zhang et al. (2017b) and Zhou et al. (2018). All the samples were stored at 4°C until further measurements.

Protein Solubility

The myosin solutions were diluted with each corresponding buffer to 10 mg/mL, followed with centrifugation at 10,000 × *g* for 20 min (Chen et al., 2018). The solubility was defined as a percentage of the protein concentration in the supernatant relative to the concentration before centrifugation. The measurements were performed in triplicate using independently prepared samples.

Surface Hydrophobicity Measurement

The surface hydrophobicity was measured using 8-anilino-1-naphthalene sulfonic acid (**ANS**) as described by Chen et al. (2014). The myosin solutions were diluted in corresponding buffers to 1 mg/mL. Subsequently, 20 μL of 15 mmol/L ANS solution (dissolved in 0.1 mol phosphate buffer, pH 7.0) was added to 4 mL of the diluted myosin solution and thoroughly stirred. After 20 min at 25°C, the fluorescence was determined with a microplate reader (Varioskan Flash, Thermo Electron Corporation) using an excitation wavelength of 380 nm and an emission wavelength of 470 nm. The surface hydrophobicity was expressed in fluorescence intensity (arbitrary units, a.u.). The measurements were performed 6 times using independently prepared samples.

Reactive and Total Sulfhydryl Groups

As described by Guo et al. (2015), the myosin solutions were diluted in corresponding buffers with or without 8 mol urea to 1 mg/mL. 50 μL of 5,5′-dithiobis-2-nitrobenzoic acid (**DTNB**) solution (10 mmol/L in 0.6 mol phosphate buffer, pH 8) was added to 4 mL of each diluted solution and incubated at 25°C for

Table 1. Salt composition of sodium-reduced myosin solutions with or without HPP.

Treatments		NaCl (mol)	KCl (mol)	K-lactate (mol)	K-citrate (mol)
0.1 MPa	C	0.4 (Na^+)	-	-	-
	K1	0.3 (Na^+)	0.1 (K^+)	-	-
	K2	0.3 (Na^+)	-	0.1 (K^+)	-
	K3	0.3 (Na^+)	-	-	0.1 (K^+)
200 MPa	HC	0.4 (Na^+)	-	-	-
	HK1	0.3 (Na^+)	0.1 (K^+)	-	-
	HK2	0.3 (Na^+)	-	0.1 (K^+)	-
	HK3	0.3 (Na^+)	-	-	0.1 (K^+)

Abbreviation: HPP, high-pressure processing.

20 min. The amount of sulfhydryl (**SH**) content was measured at 412 nm using a molar extinction coefficient of 13,600 mol^{-1} cm^{-1}. The measurements were performed 6 times using independently prepared samples.

Apparent Viscosity Measurement

Myosin solutions (25 mg/mL) were subjected to a 1 mm slit between the plates, the shear rate was increased from 1 s^{-1} to 1,000 s^{-1} (Fernández-Ávila et al., 2015), and the flow curve was obtained by using a rheometer (Discovery HR-3, TA Instruments Co.) with a cone and plate geometry of 40 mm diameter with 2° cone angle. The measurements were performed in triplicate using independently prepared samples.

Hydration Capacity

Low-field nuclear magnetic resonance (**LF-NMR**) spin–spin relaxation was measured using the method described by Zhou et al. (2018). The transverse relaxation (T_2) determination was performed with a relaxation delay of 6 s and 16 scans. The top points of 18,000 echoes were acquired using a tau of 14 μs at 32°C. The measurements were performed in triplicate using independently prepared samples.

Statistical Analysis

All data were analyzed using the General Linear Models procedure in Statistix 8.1 (Analytical Software, St. Paul, MN). Analysis of variance with Tukey's multiple comparison was used to measure the significance between different treatments ($P < 0.05$).

RESULTS AND DISCUSSION

Solubility

As shown in Figure 1, the solubility of nonpressurized myosin was not varied significantly with the replacement of sodium chloride by KCl (**K1**) ($P > 0.05$). The partial replacement of Na^+ by K^+ with the same accompanying anion (Cl^-) showed a similar solubilizing effect. However, the partial replacement by K-lactate and K-citrate (**K2** and **K3**) resulted in a dramatic decrease in the myosin solubility ($P < 0.05$) comparing with the control, and the K3 group was slightly higher than the K2 group ($P > 0.05$). These results were mainly attributed to the different anion types because the Na^+ was replaced by equal molar masses of K^+ in all the treated groups. It was suggested that the effects of potassium salts on myosin solubility strongly depend on the accompanying anions and that KCl was the most effective substitute in maintaining the solubility, whereas the organic K-lactate and K-citrate were detrimental to the solubility.

The HPP significantly improved the solubility of myosin ($P < 0.05$) relative to the corresponding nonpressurized samples, except HK1 (Figure 1). Of interest for this work was the extremely significant improvement of HPP-treated myosin solubility in organic salt groups of K2 and K3 (the relative increases of solubility were 263% and 425%, respectively), and furthermore, the HK3 showed a solubility similar to that of the control (Figure 1). The changes in myosin solubility of organic salt groups could be responsible for the water retention variation of chicken breast gel in previous study

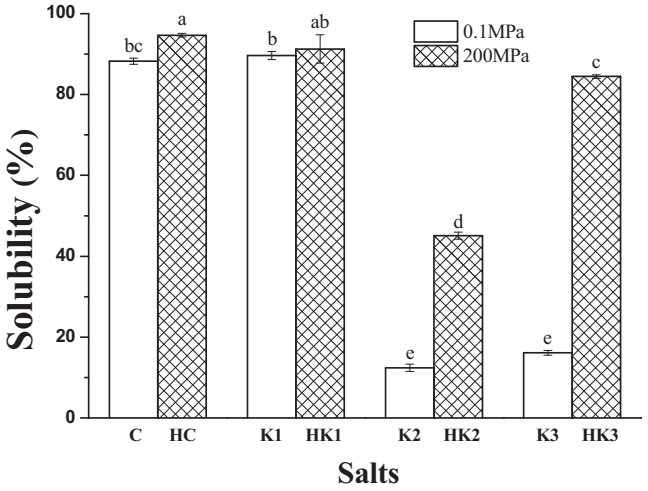

Figure 1. Solubility of pressurized chicken myosin with 3 anion types of potassium salt at 200 MPa for 10 min. [a–e]Means with the various letters are significantly different ($P < 0.05$; n = 3). C: Myosin with 0.4 mol NaCl; K1: Myosin with 0.3 mol NaCl + 0.1 mol KCl; K2: Myosin with 0.3 mol NaCl + 0.1 mol K-lactate; K3: Myosin with 0.3 mol NaCl + 0.1 mol K^+ of K-citrate; HC: Myosin with 0.4 mol NaCl at 200 MPa for 10 min; HK1: Myosin with 0.3 mol NaCl + 0.1 mol KCl at 200 MPa for 10 min; HK2: Myosin with 0.3 mol NaCl + 0.1 mol K-lactate at 200 MPa for 10 min; HK3: Myosin with 0.3 mol NaCl + 0.1 mol K^+ of K-citrate at 200 MPa for 10 min.

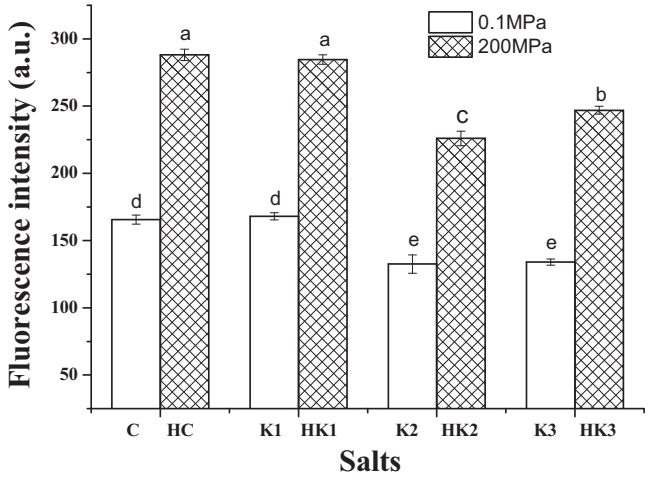

Figure 2. The surface hydrophobicity of pressurized chicken myosin with 3 anion types of potassium salt at 200 MPa for 10 min. [a–e]Means with the various letters are significantly different ($P < 0.05$; n = 6). C: Myosin with 0.4 mol NaCl; K1: Myosin with 0.3 mol NaCl + 0.1 mol KCl; K2: Myosin with 0.3 mol NaCl + 0.1 mol K-lactate; K3: Myosin with 0.3 mol NaCl + 0.1 mol K^+ of K-citrate; HC: Myosin with 0.4 mol NaCl at 200 MPa for 10 min; HK1: Myosin with 0.3 mol NaCl + 0.1 mol KCl at 200 MPa for 10 min; HK2: Myosin with 0.3 mol NaCl + 0.1 mol K-lactate at 200 MPa for 10 min; HK3: Myosin with 0.3 mol NaCl + 0.1 mol K^+ of K-citrate at 200 MPa for 10 min.

(Zhou et al., 2018). Generally, the degree of protein solubilization in an aqueous medium is the result of electrostatic and hydrophobic interactions between protein molecules (Chantarasuwan et al., 2011). High-pressure processing can induce the breakdown of salt bonds and hydrophobic interactions, resulting in unfolding of the protein (Cheftel and Culioli, 1997) and thus changing the intramolecular and intermolecular noncovalent interactions. The present result was most likely that the myosin with 3 anion types of potassium salts before HPP had already induced different molecular interactions, leading to different extents of modifications of noncovalent interactions during HPP and then contributing to different changes of myosin solubility.

These results suggested that HPP could compensate for the NaCl replacement–induced decreases in myosin solubility although the substitution of NaCl by organic potassium salts was inferior to that by KCl, and the compensated magnitude depended on the anion type of the potassium salts.

Surface Hydrophobicity

As shown in Figure 2, the replacement of NaCl by KCl did not affect ($P > 0.05$) the surface hydrophobicity of nonpressurized myosin, while the addition of K-lactate and K-citrate induced a significant decrease ($P < 0.05$). These results suggested that the NaCl and KCl cause a similar degree of protein molecular unfolding, and K-lactate and K-citrate cause a relatively lower degree of protein molecular unfolding, which were in agreement with the lower solubility (Figure 1) of myosin.

The HPP significantly increased the surface hydrophobicity of 4 groups, and the increases varied with the anion types (Figure 2). Generally, the hydrophobic groups are previously buried in the interior of the native proteins (Ikeuchi et al., 1992; Ko et al., 2003), and the myosin polymers are bound by their tails to form filamentous aggregates. However, HPP could dissociate the native aggregation that was bound by myosin tails and simultaneously promote a new type of aggregation that was bound by the head portion (Chapleau et al., 2004). The hydrophobic residues of both the C and K1 groups were exposed to a higher extent before HPP when compared with K2 and K3 groups, which suggested that the initially exposed hydrophobic residues could promote the aggregation of myosin via hydrophobic interaction during pressurization while the unfolding of the protein structure occurred simultaneously, and thus, the overall impact on the myosin solubility was less evident in the C and K1 groups than in the organic salt groups (Figure 1). The exposure of hydrophobic groups in K3 was more significant than that in K2 during HPP, indicating a greater extent of unfolding of myosin and leading to a higher solubility (Chantarasuwan et al., 2011). HPP could also improve the hydrophilic ability of proteins via the domination over the simultaneous strengthening of hydrophobicity (Xue et al., 2018) and thus induced an enhanced solubility.

Reactive and Total SH Groups

As shown in Figure 3A, the effect of KCl on reactive SH content was insignificant ($P > 0.05$), while the K-lactate and the K-citrate induced a significant decrease when compared with the control ($P < 0.05$), which was consistent with the changes in surface hydrophobicity (Figure 2). However, no significant changes ($P > 0.05$) were observed in the total SH content of nonpressurized samples when NaCl was substituted by one of three salts (Figure 3B), indicating that the salt substitution did not cause the disruption or formation of disulfide bonds (Guo et al., 2015).

Regardless of the salt substitute incorporation, HPP induced a significant increase in the reactive SH content ($P < 0.05$), indicating exposed SH groups on the surface of the protein and unfolding of the protein structure (Figure 3A). Notably, the extent of increase of reactive SH was more obvious in organic salt groups than in C/K1 groups during HPP, possibly due to the intramolecular and intermolecular disulfide bonds that formed during the pressurization in C/K1 groups. Furthermore, a significant decrease ($P < 0.05$) of total SH was found in the C and K1 groups after HPP, whereas no significant effect ($P > 0.05$) was observed in the K2 and K3 groups (Figure 3B). The volume-decreasing effect of HPP could shorten the distances of SH groups, facilitating the interaction between the reactive SH groups, thereby enhancing the formation of intermolecular disulfide bonds through the SH/SS interchange reaction (Cheftel and Culioli, 1997), and resulting in decreased total SH contents. However, the HPP-induced exposure of sulfhydryl groups was dominant over the simultaneous formation of intermolecular disulfide bonds, which

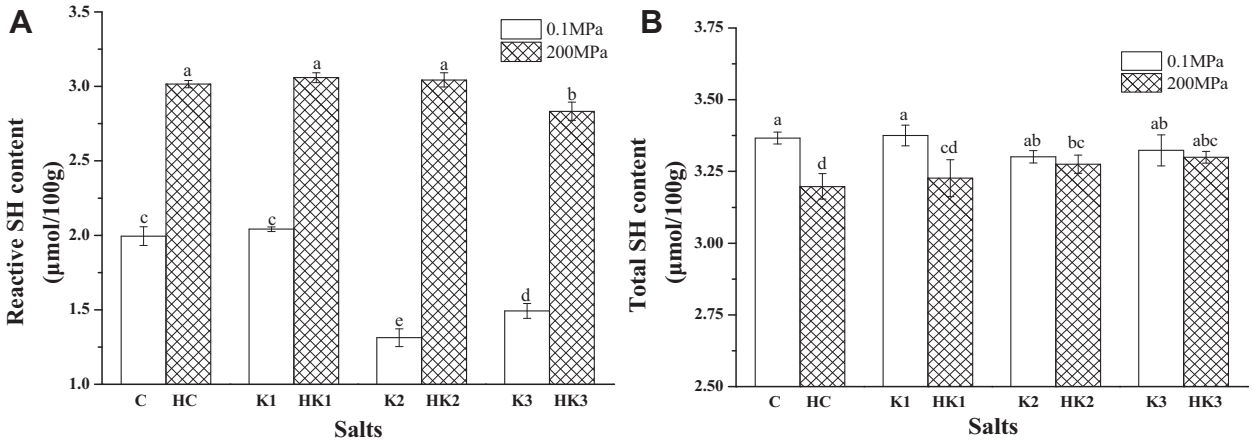

Figure 3. Reactive (A) and total (B) sulfhydryl (SH) groups of pressurized chicken myosin with 3 anion types of potassium salt at 200 MPa for 10 min. [a–e]Means with the various letters are significantly different ($P < 0.05$; n = 6). C: Myosin with 0.4 mol NaCl; K1: Myosin with 0.3 mol NaCl + 0.1 mol KCl; K2: Myosin with 0.3 mol NaCl + 0.1 mol K-lactate; K3: Myosin with 0.3 mol NaCl + 0.1 mol K^+ of K-citrate; HC: Myosin with 0.4 mol NaCl at 200 MPa for 10 min; HK1: Myosin with 0.3 mol NaCl + 0.1 mol KCl at 200 MPa for 10 min; HK2: Myosin with 0.3 mol NaCl + 0.1 mol K-lactate at 200 MPa for 10 min; HK3: Myosin with 0.3 mol NaCl + 0.1 mol K^+ of K-citrate at 200 MPa for 10 min.

thus increased reactive SH contents in the 4 types of pressurized myosin, especially in the HK2 and HK3 groups (Figure 3A). These results suggested that HPP could induce a greater extent of unfolding of myosin with organic salts, hence resulting in a more significant increase of myosin solubility in the K2 and K3 groups than K1 groups (Figure 1).

Apparent Viscosity

As presented in Figure 4, the viscosity of nonpressurized suspensions decreased with increased shear rate, suggesting a shear-thinning or pseudoplastic behavior of the suspensions (Song et al., 2013). The KCl did not affect the viscosity of the myosin suspension, whereas the K-lactate and the K-citrate induced much lower viscosities at all shear rates comparing with the control. Both the K2 and K3 groups showed extremely low solubilities (Figure 1), resulting in a complete separation between the large protein aggregates and the water phase and then contributing to a low viscosity.

The pressurized sample displayed a much lower viscosity than nonpressurized (Figure 4), suggesting that the HPP could present sufficient energy to disrupt noncovalent interactive forces (Sikes et al., 2009) as hydrophobic interactions (Figure 2) and impair the protein-protein interactions (Fernández-Ávila et al., 2015; Xue et al., 2017). In addition, the present low viscosity is possibly relative to the stronger interactions between pressurized myosin and solvent (Xue et al., 2017). Hence, the HPP could lead to a decrease in viscosity and an increase in the solubility of myosin.

Hydration Capacity

As shown in Table 2, the LF-NMR transverse (T_2) relaxation times and the corresponding populations (P_2) were observed in most of the myosin solutions. Furthermore, the relaxation components, T_{2b} (0.1–10 ms), T_{21} (10–100 ms), and T_{22} (100–1,000 ms), respectively, refer to bound, immobilized, and free water, and the longer relaxation time indicates more loose bounding of the water species to the macromolecules (Zhou et al., 2018).

As far as the salt substitutions were concerned, no obvious changes were observed in T_2 and P_2 between C and K1 groups ($P > 0.05$), suggesting that the effect of KCl substitution on the hydration capacity was unapparent. By contrast, longer relaxation times of T_{2b} and higher corresponding proportions of this water component (P_{2b}) were observed in K2 and K3 groups when compared with the control (Table 2). The T_{2b} of lower relaxation times (<10 ms) is attributed to the water of hydration (Shao et al., 2016). The increase of the T_{2b}

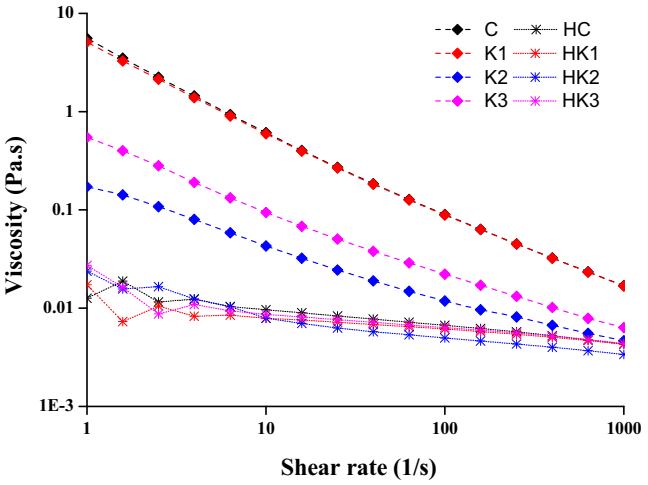

Figure 4. Apparent viscosity of pressurized chicken myosin with 3 anion types of potassium salt at 200 MPa for 10 min. n = 3. C: Myosin with 0.4 mol NaCl; K1: Myosin with 0.3 mol NaCl +0.1 mol KCl; K2: Myosin with 0.3 mol NaCl +0.1 mol K-lactate; K3: Myosin with 0.3 mol NaCl +0.1 mol K^+ of K-citrate; HC: Myosin with 0.4 mol NaCl at 200 MPa for 10 min; HK1: Myosin with 0.3 mol NaCl +0.1 mol KCl at 200 MPa for 10 min; HK2: Myosin with 0.3 mol NaCl + 0.1 mol K-lactate at 200 MPa for 10 min; HK3: Myosin with 0.3 mol NaCl +0.1 mol K^+ of K-citrate at 200 MPa for 10 min.

Table 2. LF-NMR transverse (T_2) relaxation times and corresponding populations (P_2) of pressurized chicken myosin with 3 anion types of potassium salt under 200 MPa for 10 min.

Sample [1]	T_{2b} (ms)	P_{2b} (%)	T_{21} (ms)	P_{21} (%)	T_{22} (ms)	P_{22} (%)
C	0.174 ± 0.024^{b}	0.048 ± 0.004^{d}	-	-	613.591 ± 0.000^{c}	99.952 ± 0.004^{a}
HC	0.449 ± 0.064^{a}	$0.104 \pm 0.003^{c,d}$	-	-	811.131 ± 0.000^{a}	99.896 ± 0.003^{a}
K1	0.173 ± 0.038^{b}	0.024 ± 0.010^{d}	-	-	613.591 ± 0.000^{c}	99.871 ± 0.191^{a}
HK1	0.248 ± 0.000^{b}	0.535 ± 0.131^{a}	-	-	811.131 ± 0.000^{a}	99.465 ± 0.131^{b}
K2	0.554 ± 0.114^{a}	$0.164 \pm 0.046^{b,c,d}$	28.666 ± 3.991^{a}	0.550 ± 0.099^{a}	674.850 ± 53.052^{b}	99.286 ± 0.137^{b}
HK2	0.299 ± 0.025^{b}	$0.426 \pm 0.258^{a,b}$	-	-	705.480 ± 0.000^{b}	$99.574 \pm 0.258^{a,b}$
K3	0.299 ± 0.025^{b}	$0.371 \pm 0.085^{a,b,c}$	-	-	613.591 ± 0.000^{c}	$99.629 \pm 0.085^{a,b}$
HK3	0.249 ± 0.035^{b}	$0.330 \pm 0.090^{a,b,c,d}$	-	-	705.480 ± 0.000^{b}	$99.670 \pm 0.090^{a,b}$

[a–d]Means with different letters in the same column are significantly different ($P < 0.05$; n = 3).
Abbreviation: LF-NMR, low-field nuclear magnetic resonance.
[1]C: Myosin with 0.4 mol NaCl; K1: Myosin with 0.3 mol NaCl +0.1 mol KCl; K2: Myosin with 0.3 mol NaCl +0.1 mol K-lactate; K3: Myosin with 0.3 mol NaCl +0.1 mol K^+ of K-citrate; HC: Myosin with 0.4 mol NaCl at 200 MPa for 10 min; HK1: Myosin with 0.3 mol NaCl + 0.1 mol KCl at 200 MPa for 10 min; HK2: Myosin with 0.3 mol NaCl +0.1 mol K-lactate at 200 MPa for 10 min; HK3: Myosin with 0.3 mol NaCl +0.1 mol K^+ of K-citrate at 200 MPa for 10 min.

value implied an increased molecular mobility of the bound-water and a lower hydration state, especially for the K2 group. However, the increased P_{2b} appeared inconsistent with the lower hydration state and solubility observed in K2 and K3. This phenomenon was possibly resulted from the strong preference of water molecules to hydrate the lactate and citrate anions rather than myosin by donating H-bonds to them, and thus interfering the protein-water interaction, because the presence of highly hydrated anions may interfere with the "salt-in" effect of cations (Wu et al., 2016).

After pressurizing at 200 MPa, the C and K1 groups revealed higher T_{2b} values and relative proportions (P_{2b}) (Table 2), suggesting that the bound-water became more mobile, but the content of the hydrated water was simultaneously increased. These results were in accordance with the improvement of myosin solubility in HC and HK1 groups at 200 MPa (Figure 1). Furthermore, the T_{2b} values of the HK2 and HK3 groups were less than those of the corresponding unpressurized groups, suggesting an increased hydration capacity in the pressurized myosin colloid solutions with K-lactate and K-citrate. The HPP-induced volume-decreasing effect can also increase the ionic product $[H^+] \times [OH^-]$ of water, including protein-bound water (Cheftel and Culioli, 1997). And HPP-induced conformational fluctuations provide pathways for water to penetrate into the interior of the native protein, and the water exchange between the protein interior and bulk solvent can be enhanced (Boonyaratanakornkit et al., 2002). These implied that more intermolecular H-bonds were formed between myosin and water, rather than lactate or citrate anions and water after HPP, resulting in increased hydration capacity of myosin, and thus reduced the viscosity (Figure 4) and improved the solubility of HK2 and HK3.

CONCLUSION

The HPP of 200 MPa could compensate for the NaCl replacement–induced decreases in myosin solubility, and this effect depended on the type of anion that accompanied the potassium salts. The lower hydrophobic group and reactive SH content of nonpressurized myosin with K-lactate or K-citrate possibly contributed to the aggregation of myosin molecules compared with the KCl group and thus decreased solubility. The HPP could significantly reduce apparent viscosity and increase the surface hydrophobicity, reactive SH, and hydration capacity of myosin with K-lactate and K-citrate, indicating the unfolding of myosin with a disruption of protein–protein interactions and the strengthening of protein–water interactions and thus improving the solubility of myosin with organic K-lactate or K-citrate.

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The authors declare no competing financial interest.

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