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RHEOLOGICAL AND TEXTURAL PROPERTIES OF SODIUM REDUCED SALT SOLUBLE MYOFIBRILLAR PROTEIN GELS CONTAINING SODIUM TRI-POLYPHOSPHATE

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Abstract

The effect of partial replacement of NaCl (50%) with KCl in the presence of sodium tri-poliphosphate (STPP) on the cooking loss (CL), water holding capacity (WHC), rheological and textural properties of salt soluble myofibrillar protein (SSMP) gels was investigated. KCl substitution, either alone or in combination with STPP, was found worse than NaCl alone in terms of elasticity of the gels (G'). Both KCl and STPP reduced the CL of the gels. While the gels with replacement of NaCl with KCl had lower (P<0.05) WHC, addition of STPP to this gels gave rise to similar (P>0.05) WHC to only NaCl added gels. Substitution of NaCl with KCl resulted in a decrease in hardness of gels whereas STPP addition improved the hardness. Results obtained from the present study suggest that substitution 50% of NaCl with KCl in presence of STPP would be a sound salt reduction alternative in meat systems.

Keywords: Rheology; Meat proteins; Salt reduction

Practical Applications

Salt is one of the most crucial ingredients affecting meat products functionality. However, recent research has focused on reducing salt in meat products due to its association with some health problems such as hypertension. Reducing sodium chloride (NaCl) decreases extractability of salt soluble myofibrillar proteins (SSMP), thus, negatively affects functional properties of the meat systems. One of the approaches in reducing salt is to replace NaCl with other chloride salts, the most common of which is potassium chloride (KCl). This study focused on determining the effect of partially substituting of NaCl with KCl in the presence of sodium tri-polyphosphate (STPP) on some functional characteristics of meat SSMP gels. Results indicated that 50% NaCl reduction using KCl and STPP could be achieved with an improvement of textural and functional properties in meat gels. Future research should focus on combined effects of salt substitutes on the rheological and textural characteristics of meat gel systems.

I. INTRODUCTION

Interest in healthier foods by today's consumers has led meat technologists to develop new functional products or to improve formulations of existing ones. In this regard, reducing sodium or salt in food products has drawn tremendous attention due to public awareness and proven epidemiological studies indicating that dietary sodium intake causes or aggravates health problems such as stroke, coronary heart disease, kidney disease and cancer (Antonios and MacGregor 1997).

Processed meats fall into the group of food products which have relatively high salt content. It has been reported that approximately 20-30% of dietary sodium chloride intake in western countries arises from processed meats (Barbut and Mittal 1989; Ruusunen and Puolanne 2005).

For this reason, various attempts have been made to develop reduced salt meat products with acceptable quality characteristics. Sodium chloride, as an essential ingredient for processed meat formulations, plays an important role in shelf life, taste and textural characteristics of the final product. Sodium chloride contributes to the functionality of meat proteins by extracting salt soluble myofibrillar proteins (SSMP), thus providing good emulsion, improving the gelation and binding properties of SSMP resulting from the interaction between meat proteins and chloride ions (Ruusunen and Puolanne 2005). Therefore, when the content of sodium chloride is reduced to a certain level, the negative impact not only raises the sensory and microbiological properties of the products, but there is also a decrease in extractability of myofibrillar proteins, resulting in poor functionality (Totosaus and Perez-Chabela 2009).

Myofibrillar proteins in meat systems play an essential role in textural properties mainly by contributing to gelation characteristics. In gelation of meat proteins, a three-dimensional cross-linked network structure is formed after partial unfolding or denaturation of proteins, and subsequent aggregation of myosin heads through disulfide bounds formation and helix-coil transitions of myosin tails, resulting in significant changes in rheological properties of the system (Samejima *et al.* 1982; Fretheim *et al.* 1986; Sharp and Offer 1992; Xiong and Blanchard 1993; Sun and Holley 2011).

Rheological characteristics of meat proteins depend on several factors, such as protein type and concentration, type and source of muscle, ionic strength, pH, heating rate and temperature, and additives improving gel formation (Sun and Holley 2011). One of the most important factors influencing gel formation is ionic strength, which can be ensured by the use of sodium chloride at approximately 2-3% level in the case of comminuted meat products, which is necessary for myofibrillar protein solubilization, and thus development of functional properties like water binding capacity of the final product (Xiong and Brekke 1991; Sun and Holley 2011).

One approach in reducing sodium chloride in the formulation of meat products is to replace it with other chloride salts such as KCl, LiCl, $CaCl_2$ or $MgCl_2$. Among these potential chloride salts, KCl has been reported to be the most relevant alternative to sodium chloride in terms of protein solubility, protein functionality, water binding capacity and flavor. Because it imparts bitter taste notes when used in excessive amounts, KCl concentrations lower than 50% in meat formulations has been recommended for sodium chloride replacement (Ruusunen and Puolanne 2005). One other possible alternative in reducing sodium chloride in meat systems is the use of alkali phosphates, which have been used as curing agents to improve protein extraction, resulting in better protein functionality with subsequent improvement of textural properties of the products (Robe and Xiong 1992; Price 1997; Lampila and Godber 2002). Thus, the aim of the present study was to evaluate the effects of partial substitution of NaCl with KCl in the presence of sodium tri-polyphosphate (STPP) on the textural, rheological and water binding properties of SSMP gels from beef *Semimembranosus* muscles.

2. MATERIALS AND METHODS

2.1 Material

Beef *semimembranosus* muscles were purchased from a local butcher shop in Oxford, UK in different weeks for the two replications. The beef muscles were trimmed of visible fat and connective tissue, cut into small pieces (5x10 mm), and vacuum-packed with polyethylene bags in the laboratory. The beef was then transferred to the lab in refrigerated containers and kept at -18°C for further analysis, for no more than one week.

2.2 Extraction of Salt Soluble Myofibrillar Protein

Salt soluble myofibrillar proteins (SSMP) were extracted according to the method described by Chen *et al.* (2007), with slight modifications. Beef pieces prepared as described above were thawed at 4°C overnight, and blended with three volumes of isolation buffer (0.5 M KCl, 17.8 mM $Na_5P_3O_{10}$, pH 8.7) by using a laboratory blender for 60 s at high speed. The slurry was kept at 4°C for 1 h, followed by centrifugation at 4°C for 30 min at 10.000 rpm using a J2-21 Beckman centrifuge (USA-Minnesota). After filtering the protein extract with three layers of cheesecloth, the filtrate was diluted with distilled water for precipitation of proteins. To collect the proteins, the mixture was centrifuged at 4°C for 15 min at 10.000 rpm. Protein content was determined by the micro Kjeldahl method (AOAC 2000). Nitrogen content was converted to protein, by multiplying by 6.25. Protein concentration of the extract was adjusted to 8% using the same isolation buffer.

2.3 Preparation of SSMP Gels

The extracted SSMP solution was diluted with the isolation buffer to attain 5% protein content and divided into 6 equal parts for preparation of the gel samples for the treatment groups one of which was separated as SSMP control (T1), and it was formulated to contain 3% NaCl

(T2) as generally used in regular comminuted meat products. 1.5 % KCl added to reduce the 50% of NaCl (T3). As seen on Table 1 for the other three groups, 0.4% STPP added to combination of each (T4; T5; T6). SSMP from each treatment group was kept at 4°C overnight, and then stuffed into plastic tubes (19 mm in diameter), followed by gelation at 80°C for 30 min in a water bath (Sun *et al*. 2012). The gels were removed from water bath and cooled in cold water for 15 min, then stored at 4°C overnight. These gels were used to determine water holding capacity and cooking loss measurements.

2.4 Dynamic Rheological Measurements

Dynamic rheological measurements were performed on protein extracts using Bohlin Gemini200 Rheometer. A parallel plate, 55 mm in diameter was used with a 1 mm gap size. Single frequency at 1 Hz was used for heating the samples from 25 to 75°C at 2°C /min heating rate. Data were collected by Bohlin software and monitored during analysis at each temperature. Viscoelastic and gelation properties (storage modulus G', and loss modulus G") of duplicate SSMP mixtures were determined.

2.5 Cooking Loss

To determine cooking loss, the SSMP extracts were weighed in the plastic tubes before gelation (initial weight). After gelation conducted as mentioned above,duplicate cooled gelswere stored at 4°C over night, removed from plastic tubes, and dried with filter paper followed by measuring the final weights. Cooking loss (CL) was calculated as a percentage based on the initial weight (Cong-Gui *et al.* 2006).

2.6 Water Holding Capacity

Triplicate gel samples (19 mm in diameter, 10 mm in height) were weighed into centrifuge tubes with absorbent cotton on the bottom, and centrifuged at 3000 rpm at 4°C for 10 min to determine water holding capacity (WHC) according to the method reported by Ma *et al.* (2012). WHC was expressed as the ratio of centrifuged gel weight to the initial gel weight.

2.7 Texture Profile Analysis

For determination of textural characteristics, samples were cut into cylinders 10 mm in height and 19 mm in diameter, and compressed twice to 50% of their original height by cylindrical parallel plate by 0.6N trigger force at a constant speed 1 mm/s, without a delay between two compressions in an LFRA Texture Analyzer (UK- Harlow). This procedure was repeated four times for each group. TPA parameters were calculated from the force–deformation curves, and expressed as hardness (N), cohesiveness, chewiness (Nmm) and springiness (mm) of the gel samples (Pietrasik 2003).

2.8 Statistical Analysis

Data from two replications were analyzed with analysis of variance procedure of SAS system 9 (SAS, 2002), and mean separation was conducted using Duncan's Multiple Comparison test at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1 *Dynamic Rheological Properties*

Myofibrillar proteins, mainly myosin and actin, are responsible for viscoelastic gel formation in meat products (Westphalen *et al.* 2005). During heat induced gelation of SSMP, actin and myosin aggregate and exhibit three dimensional viscoelastic behavior as described by dynamic modulus, including both storage modulus (G') and loss modulus (G''), which indicate elasticity and viscosity of the material, respectively (Chen *et al.* 2007; Li 2008). In the current study, in order to observe the effect of NaCl, KCl and STPP addition on the gelation properties of the reduced-salt SSMP gels, dynamic modulus (G' values obtained at the end of heating at 75°C) was determined, and representative rheograms for each gel group are presented in Figure 1. The highest G' was observed in gels with the addition of 3% NaCl (T2).

Replacement of NaCl with KCl at 50% level (T3) in SSMP gels resulted in lower G' values (P<0.05) at the end of the heating process as compared to group T2, which demonstrated that addition of KCl resulted in less elastic gel than NaCl, although it was used at the same ionic strength. This lower G' value resulting from the addition of KCl could be explained by better binding of Na^+ ions to protein surface than K^+ ions, as previously mentioned by Friedman (2011) due to a weaker electric field of K^+ than that of Na^+, resulting in a lower hydrated layer on the protein surface when KCl is used in the formulation (Lian *et al.* 2002).

Similar to NaCl, phosphates also exhibit improved elasticity of meat gels with a synergistic effect on gel strength when both are used together (Fernandez-Martin *et al.* 2002; Lampila and Godber 2002; Sun and Arntfield 2011). Ionic strength has a crucial effect on the gelation ability of proteins as a result of its effect on the solubility of myofibrillar proteins, which could be attained by the addition of phosphates at certain concentrations. At lower ionic strength, better gel networks are formed in meat systems with more rigid and fine-stranded gels than those in higher ionic strength (Kaminer and Bell 1966; Hermansson *et al.* 1986; Yang *et al.* 2007; Sun and Arntfield 2011). Fretheim *et al.* (1986) reported higher G' when NaCl was used at low ionic strength (0.2M) and lower G' when it was used at high ionic strength (0.6M) in heat induced gels of myosin suspensions/solutions from red and white bovine muscles. Accordingly, Boyer *et al.* (1996) observed an increase in rigidity on rabbit muscle myofibrils when the ionic strength was lowered from 0.6 to 0.2 M KCl. Liu and Xiong (1997) determined the gelation characteristics of chicken myofibrillar protein gels formed under various NaCl concentrations (0.3, 0.4 and 0.6M) in the presence of TPP (tripolyphosphate), and found that TPP exhibited improved gelation of myofibrillar protein at low ionic strengths, i.e., 0.3-0.4 M NaCl, which equals approximately 1.5% NaCl; however, at 0.6M NaCl concentration (approximately 3% NaCl), the gelling ability was decreased. In agreement with the results of the aforementioned study, lower G' was determined in the group T5 containing 3% NaCl in the presence of STPP than in the group T2 with the addition of only 3% NaCl in the current study (P<0.05). Nevertheless, there was no significant effect of STPP on gelation properties of the SSMP gels formed with 50% KCl replacement to NaCl (T3). It is apparent that STPP did not have a detrimental effect on gelation ability when it was used together with KCl.

3.2 *Cooking Loss*

SSMP gels containing 3% NaCl (T2) obviously had lower cooking loss (P<0.05) than control (T1), as shown in Figure 2. It was previously reported that cooking loss is reduced due to greater water binding ability in the protein network as a result of increased protein extractability with increases in NaCl concentration (Barbut *et al.* 2009). Cooking loss appeared to be lower (P<0.05) in the group T3 with replacement of 50% of NaCl with KCl than in the group T2. Moon *et al.* (2008) noted that partial replacement of 40% NaCl with KCl caused lower cooking loss than the 100% NaCl in pork patties, which is in agreement with the result of the current study. In a previous study by Alino *et al.* (2009) on the effect of partial substitution of sodium with different chloride salts including potassium chloride on the salting kinetics of dry cured hams, a decrease in water loss was observed in the presence of K^+ ion.

STPP addition at 0.4% level to 3% NaCl added group (T5) resulted in lower CL than 3%NaCl added group (T2), as expected. This CL lowering effect of STPP could be explained by the boosting effect of phosphates on water binding characteristics of meat products as a result of increased ionic strength, in which case, due to the freed negatively charged sites in proteins, proteins are able to bind more water (Ruusunen and Puolanne 2005). In the gels with replacement of NaCl with KCl, addition of 0.4% STPP (T6) did not cause a significant change in cooking loss (P>0.05). In 3% NaCl added groups, incorporation of STPP (T5) improved cooking loss by significantly (P<0.05) reducing it as compared to the group T2 with only 3% NaCl addition.

3.3 *Water Holding Capacity*

Water holding capacity (WHC) values for SSMP are presented in Figure 3. The lowest WHC (P<0.05) was found in the control gel (T1), which did not differ (p>0.05) from the gels with the addition of only STPP. When gel groups with salt and without STPP addition compared, 3% NaCl added group (T2) had higher (P<0.05) WHC than that with 1.5% NaCl and 1.5% KCl (T3). KCl exhibited lower protein extractability than NaCl (Munasinghe and Sakai 2003). There are differences in the effects of Na^+ and K^+ ions on myosin conformation because K^+ ion possesses weaker electrical field and lower protein interaction than Na^+ ion (Palladino and Ball 1979; Lian *et al.* 2002), which could result in differences in solubility of myosin and total protein extractability (Munasinghe and Sakai 2004).

The addition of 0.4% STPP did not have a significant effect on WHC of 3% NaCl added gels (T5). On the other hand, STPP significantly improved (P<0.05) WHC of gels with 50% replacement with KCl (T6). In agreement with this result, Lee *et al.* (2012), in marinated chicken breasts, found that increased amount of KCl resulted in decreased WHC, and 0.4% STPP addition overcame the detrimental impact of KCl on WHC. Similarly, Singh and Sharma (2009) reported that when 2% NaCl was replaced with KCl in chicken meat emulsions up to 1% level with 0.5% STPP improved water holding capacity of the system.

3.4 *Texture Profile Analysis*

The results for textural parameters of SSMP gels as affected by different salt addition are presented in Table 2. NaCl addition exhibited an increasing effect ($P<0.05$) on all of the measured textural characteristics (hardness, cohesiveness, chewiness, springiness) of the SSMP gels in comparison to the SSMP control (T1). Similarly, numerous research exist indicating that the textural properties of meat products are positively affected by salt addition (Gelabert *et al.* 2003; Ruusunen and Puolanne 2005; Armenteros *et al.* 2009). Replacement 1.5% of NaCl with KCl (T3) reduced the hardness of gels; however, no difference was found in other textural properties (cohesiveness, chewiness and springiness) of the gels as compared to group T2 with 3% NaCl addition. Changes in hardness or parameters in meat products are associated with differences in the water holding capacity. Lower water holding capacity results in products with lower hardness or tenderness (Desmond 2006; Horita *et al.* 2011).

Similar to findings of the current study, Gimeno *et al.* (1999) reported that replacement of NaCl with 40% of KCl resulted in reduction in hardness value of dry fermented sausage. In the contrary, Campagnol *et al.* (2011) reported that partial replacement 50% of NaCl with KCl in fermented cooked sausage had no effect on TPA parameters such as hardness, springiness and cohesiveness ($P>0.05$). These differences between the various studies and the current study might be due to differences in the experimental material used.

Salt is used in meat systems mainly to solubilize myofibrillar proteins, thus increasing water binding capacity, and to improve texture (Desmond 2006). Textural properties are influenced by the gelation ability of myofibrillar proteins (Westphalen *et al.* 2005). In the current study, addition of STPP (T5 and T6) to the gels containing NaCl and KCl, had no significant effect ($P>0.05$) on the TPA parameters in comparison to groups T2 and T3.

4. CONCLUSIONS

In the present study, 50% substitution of NaCl by using KCl with or without STPP resulted in significant reduction in elasticity of the gels. On the other hand, NaCl reduction with KCl in the presence of STPP did not changed the water binding properties, and these effects were also noticeable in textural properties, mainly in the hardness of gels. These results suggest that NaCl reduction at 50% level by using KCl and STPP could be achieved with an improvement of textural and functional properties in meat gels. Such meat products with added KCl to reduce NaCl in the presence of STPP could be an alternative for healthier diets without textural defects.

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Table 1

NaCl, KCl AND STPP ($Na_5P_3O_{10}$) CONTENTS OF EACH TREATMENT.

Group	Treatment
T1	SSMP
T2	SSMP + 3% NaCl
T3	SSMP + 1.5% NaCl+1.5% KCl
T4	SSMP + 0.4% STPP
T5	SSMP + 3% NaCl + 0.4%STPP
T6	SSMP + 1.5% NaCl + 1.5% KCl + 0.4% STPP

Table 2

EFFECT OF NaCl, KCl AND STPP TREATMENT ON TEXTURAL PROPERTIES OF SSMP GELS.

Grup	Hardness	Cohesiveness	Chewiness	Springiness
T1	3.42 ±0.45[d]	0.38 ±0.01[b]	4.91 ±0.9[c]	3.78 ±0.01[c]
T2	12.95 ±0.84[a]	0.60 ±0.05[a]	33.73 ±2.18[a]	4.24 ±0.01[a]
T3	11.41±0.15[b]	0.61 ±0.05[a]	27.09 ±0.54[a]	4.05 ±0.11[ab]
T4	8.65 ±0.12[c]	0.45 ±0[b]	15.45 ±0.28[b]	3.94 ±0.08[bc]
T5	11.62 ±0.05[ab]	0.62 ±0[a]	28.24 ±0.64[a]	4.18 ±0.07[a]
T6	12.65 ±0.16[ab]	0.63 ±0.02[a]	27.29 ±4.98[a]	4.18 ±0.02[a]

T1: SSMP ; T2: SSMP+3% NaCl ; T3: SSMP+1.5% NaCl+1.5% KCl ; T4: SSMP + 0.4% STPP; T5: SSMP+3%NaCl+0.4%STPP; T6: SSMP+1.5% NaCl+1.5% KCl+0.4% STPP.

Values are presented for two replications as means with standard error. a-d: For each attribute, means within a column (between groups) not having a common superscript letter are statistically different (P<0.05).

Figure Captions

Figure 1 Representative rheograms illustrating the storage modulus (G') of SSMP prepared with different levels of NaCl, KCl and sodium tri-polyphosphate (STPP) during heating (25 to 75°C).

Figure 2 Cooking loss (CL) of SSMP gels as function of NaCl, KCl and STPP addition.

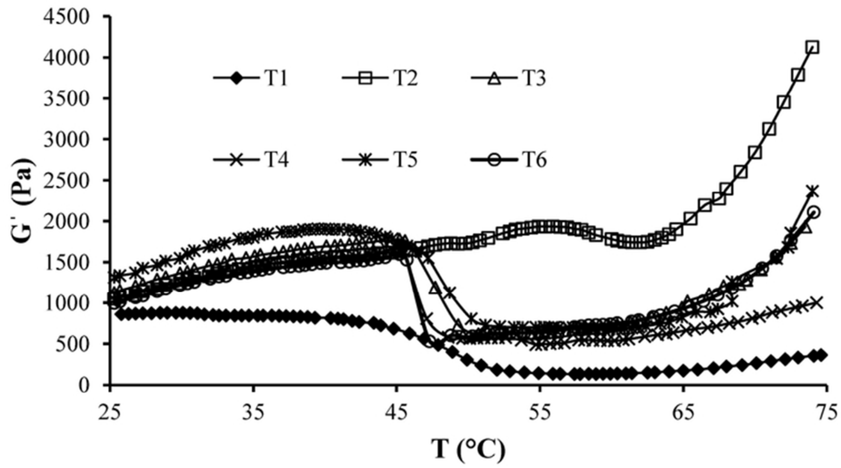
T1: SSMP ; T2: SSMP+3%NaCl ; T3: SSMP+1.5%NaCl+1.5%KCl ; T4 : SSMP+0.4%STPP ;T5: SSMP+3%NaCl+0.4%STPP ; T6 : SSMP+1.5%NaCl+1.5%%KCl+0.4%STPP.

Values are presented for two replications as means with standard error. [a-d] *Bars with different letters refer to statistically significant differences (P<0.05).*

Figure 3 WHC at different salt concentrations (T1, T2, T3, T4, T5, T6).

T1: SSMP; T2: SSMP+3%NaCl; T3: SSMP+1.5%NaCl+1.5%KCl; T4: SSMP+0.4%STPP; T5: SSMP+3%NaCl+0.4%STPP; T6: SSMP+1.5%NaCl+1.5%%KCl+0.4%STPP.

Values are presented for two replications as means with standard error. [a-d] *Bars with different letters refer to statistically significant differences (P<0.05).*



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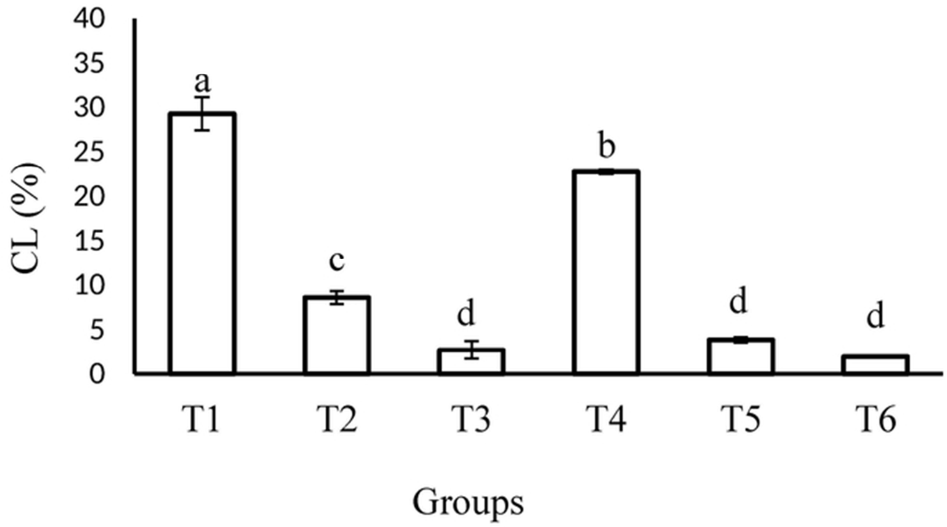

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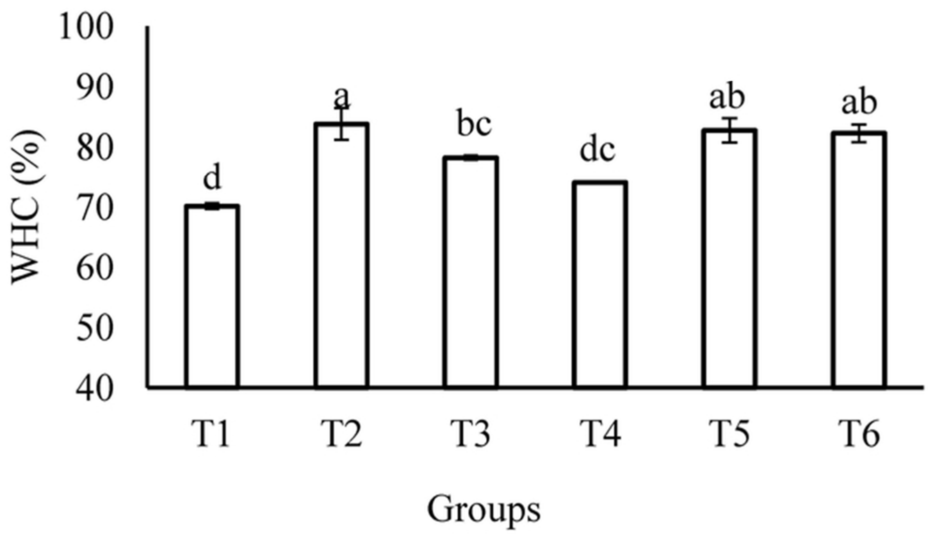

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