A Comparative Study on Effect of Egg White, Soy Protein Isolate and Potato Starch on Functional Properties of Common Carp (Cyprinus carpio) Surimi Gel

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Abstract

The effect of egg white powder (EWP), potato starch (PS) and soy protein isolate (SPI) at different levels on texture, color and sensory evaluation properties of surimi prepared from common carp was investigated. EWP was added at 1%, 2% and 3%, PS was added at 3%, 6% and 12%, and SPI was added at 10%, 20% and 30%. To evaluate the quality characteristics of the resulting surimi gel, some parameters (viscosity, gel strength, texture profile, water-holding capacity, color and sensory qualities) were analysed. The analyses indicated that the additives enhanced the functional properties of surimi gel prepared from common carp. EWP significantly improved texture properties at the greatest level (3%), whereas for color the best results came from the lowest level (1%). Conversely, PS showed its most significant effect on the surimi texture at the lowest (3%) level, but the surimi color was changed drastically at higher levels. In the case of SPI, only the lowest level (10%) did not significantly reduce the texture qualities and color of the resulting surimi gel and greater levels were detrimental to surimi gel characteristics. Finally, the best score by the panelists for overall liking was for the surimi gel containing 3% EWP. Hence, EWP can potentially improve the quality characteristics of common carp surimi gel.

Keywords: Egg white; Soy protein isolate; Potato starch; Functional properties; Surimi; Common carp

Introduction

Surimi is a Japanese word to describe the protein materials from fish flesh that has been deboned, washed with water and mixed with cryoprotectants [1,2]. Various imitations of high-priced products, such as crab legs and lobster, have been developed based on raw surimi. In nutritional value, surimi is considered a low-cholesterol, low-fat, and low-sodium ingredient that is a key to its increasing market demand [3]. Surimi is commonly obtained from white fish muscle as both whiteness and texture are considered to be the most important quality characteristics of surimi and its resulting gel products [4]. It is generally believed that to acquire a proper texture in surimi seafood products, it is necessary to enhance the fish protein gelation process [5-9], since surimi gel functional properties such as color, water-holding capacity, expressible moisture, gel strength, etc. are important aspects for acceptance of surimi-based products by consumers.

Nowadays, due to over-exploitation of some fish species or the imposition of more restricted fish catch regulations, the fish industry is trying to find alternative fish species that are suitable for making surimi [10]. Freshwater fish species production is globally increasing and could be considered as alternative fish species for surimi production as they have low market prices, acceptable mince color and texture properties. Warm-water fish species, specifically Chinese carp, which are cultured in an integrated poly-culture system, are examples. Although these species show only moderate gel-forming ability and even frozen storage affects their protein properties, they can be utilized by adapting the parameters of the gelation process [11]. In 2010, according to the statistics of the Iranian Fisheries Organization [12], total production of Chinese carp was more than 100,000 tons in Iran, of which 20,000 tons was common carp. As common carp is a bottom feeder, however, and sifts mud in seeking food particles, its flesh smells unpleasant and hence its market price is the lowest of the warm-water fish species [13].

To produce a quality surimi from freshwater fish species, it is necessary either to modify the traditional surimi-making process or to develop a specific surimi-processing technique by optimizing its key steps [14]. Furthermore, to better address the textural priorities of consumers, ingredients must be added to surimi that modifies the textural and water mobility properties of the surimi [15]. Gel properties are affected not just by minced flesh quality but also by various added ingredients. Egg white is a common additive in surimi preparation steps in order to modify the textural properties of resulting gel. The intended function of this additive is as an enzyme inhibitor to inhibit the “modori” stage (gel-softening phenomenon) during the thermal gelation process [1,2] to make the products more elastic [16].

Few studies have evaluated the effects of additives on the functional properties of common carp surimi. Hence, this study aimed to investigate the influences of egg white, potato starch and soy protein isolate at different concentrations on texture, color and sensory properties of surimi prepared from common carp.

Materials and Methods

Fish and Surimi

Fresh whole common carp fish (body length: 35.6 ± 2.3 cm; body...
weight: 700 ± 20 g) were purchased from a wholesale market in the city of Nour, Iran, and transported in ice by a refrigerated truck to the laboratory. After heading and gutting (H&G), fish were washed with cold running water, and then filleted manually. The black portion of muscle was removed and the remaining white portions used to prepare surimi. Fillets were minced in a meat mincer with a disc mesh size of 3 mm. The mince was washed with cold (10°C) distilled water three times at ratio of 1:4 (mince:water) to remove blood, mineral salts, sarcoplasmic proteins, odor components and some fats. At the final washing cycle, 0.3% NaCl was used to complete the dewatering step by manual pressing of washed fish minces in double folded cheesecloth. Prepared surimi was mixed with cryoprotectant agents (4% sugar 4% sorbitol and 0.3% sodium tripolyphosphate) and homogenized for a further 1 min. Finally, surimi was packed in blocks of 250 grams in plastic bags, frozen at -18°C and kept frozen for 1 month.

Additives

Egg white powder (EWP) was obtained from Gol Powder Golestan Co., Ltd (Agh ghala, Iran). Soy protein isolate (SPI) and potato starch (PS) were purchased from Shandong Wonderful Industrial Group Co., Ltd (China) and Qingdao ICD Biochemistry Co., Ltd (China), respectively. Technical information and quality specifications regarding these additives are shown in (Tables 1 and 2).

Preparation of surimi gel

To prepare the surimi gel, frozen surimi was chopped into small pieces of about 2×2×2 cm³ and left at ambient temperature for 1.5 h to defrost. The defrosted pieces were homogenized in a food processor for 60 s at 4-10°C to create a homogeneous paste. Ice water (to adjust the moisture content of the paste to 80 ml/100 g), 2.5% (w/w) NaCl and additives at different levels [EWP at 1%, 2% or 3%; PS at 3%, 6% or 12% and SPI at 10%, 20% or 30% (w/w)] were added separately to the common carp surimi and homogenization was continued for further 2 minutes. The paste was extruded into polyvinylidene chloride (PVC) casing with a diameter of 2.5 cm and a length of 20 cm. The stuffed casings were refrigerated at 4–5°C overnight (18 h) in order to set [17]. The next day, the surimi paste was heated for about 30 min in a hot water bath at 90 ± 2°C. The casings were cooled immediately in ice water (about 4°C) for 20 min to stop any further action of heat on the texture of the resulting surimi gels. The gels were put in plastic bags, vacuum-packed and stored for 24 h at 4-6°C for further experiments.

**Evaluation of viscosity**

To evaluate the viscosity of surimi paste before heating, 143 g of defrosted surimi from each treatment was mixed with 857 ml of 3.5% NaCl solution, homogenized for 8 min and left at room temperature for 40 min. After this, viscosity was determined with a Brookfield Tokyo Instruments Model C at 4 rps at 10°C.

**Water-holding capacity (WHC)**

WHC was determined by the method of Himonides and others (1999) [18], with slight modifications. For each treatment, three samples of 5 g each were separated. Samples were wrapped in individual Whatman filter papers (No. 41) and centrifuged at 1700x g for 30 min at 8°C. The amount of water drained from the surimi was estimated from the weight difference of the filter paper before and after centrifugation. The WHC of the common carp surimi was calculated by the following equation [18]:

\[
\text{WHC g/kg} = \frac{(1-M_w/M_s)\times 1000}{196.44}
\]

Where Mw was the mass (g) of expelled water and Ms was the initial mass (g) of the sample.

**Texture properties**

**Puncture test:** The puncture test was performed following the method described by Jafarpour and Gorczyca (2009) [19]. The gels were sliced in pieces 25 mm long and equilibrated to room temperature before puncture tests. Puncture tests were performed using a texture analyzer (Stable Micro Systems Ltd., Surrey, United Kingdom) equipped with a spherical head stainless steel probe (Ø=5 mm). The cell load was 25 kg and the crosshead speed was 60 mm/min. Gel strength calculation was based on breaking force (g) and breaking distance (mm). All evaluations were carried out using the mean of three replicates for each treatment.

**Texture profile analysis (TPA):** TPA was performed as described by Jafarpour and Gorczyca (2009). The kamaboko gel samples (height of 25 mm and diameter of 25 mm) were placed on the flat plate of a texture analyzer and axially compressed by a cylindrical plunger (Ø=50 mm) set to a cell load of 25 kg at a deformation rate of 60 mm/min. Each sample was compressed twice. Parameters measured were hardness (N), cohesiveness, adhesiveness (N/s), gumminess and elasticity. All determinations were based on the mean of three replicates for each treatment.

**Determination of whiteness**

Colorimetry of surimi treatments were performed by application of a Hunter Colorimeter, Model RT 450, in which color parameters such as L* (lightness), a* (redness/greenness) and b* (yellowness/
Pacific whiting. They showed that different fish surimi gels responded differently, depending on the nature of the fish proteins. For instance, they reported that the application of 650 MPa without additives on Alaska Pollock surimi gel and 400 MPa with egg white (EW) on Pacific whiting surimi gel resulted in the highest WHC. However, the Alaska Pollock surimi gels treated at 400 MPa without additives and with EW, alone or in combination with PS, showed a significant reduction in WHC compared with heated gels and pressurized gels at 650 MPa. By contrast, when PS was added to Pacific whiting surimi gels, WHC increased significantly, as in the common carp surimi gel studied here.

**Sensory evaluation**

The hedonic method described by Runglerkeringkrai and others (2008) [21] was used with slight modifications; 30 untrained panelists used a nine-point hedonic scale to evaluate color, odor, flavor, texture and overall popularity.

**Statistical analysis**

To evaluate the mean data from different treatments, a one-way ANOVA test was used by the application of SPSS software Version 15.0. Sensory evaluation data were analyzed by a Mann-Whitney U test. In order to determine the significance of differences between means, an LSD test was used at a confidence level of 95%. The level of significance used in all tests was p<0.05.

**Results and Discussion**

**Influence of additives on viscosity**

The effect of adding different levels of EWP, PS and SPI into the surimi paste is shown in (Table 1). The viscosity of the surimi paste was significantly affected after the addition of additives. In the surimi sample with no additives (control), the viscosity was 25800 Pa.s. Increasing levels of EWP (1%, 2% and 3%) improved the viscosity by 26%, 28% and 34% respectively. The greatest viscosity belonged to the surimi sample with 3% EW; however, there was no significant difference between 1% and 2% EW. The addition of PS increased the surimi viscosity compared with the control, but increasing levels of PS decreased the resulted viscosity significantly from about 3382 Pa.s at 3% PS to about 2783 at 12% PS, with no significant difference between 3% and 6% PS. Adding 10% SPI had no significant effect on viscosity, but the addition of 20% and 30% SPI reduced viscosity by 10.5% and 17.6% compared with the control. Therefore, it is concluded that among different additives at different concentrations, EWP showed the most affinity with the fish protein in forming more protein-protein and water-protein linkages, resulting in higher viscosity, when added to common carp surimi paste.

**Water-holding capacity (WHC)**

The influence of various concentrations of EWP, PS and SPI on WHC is shown in (Table 2). EW treatment significantly improved the WHC of samples compared to the control treatment. Similar to the viscosity results, the rate of improvement in WHC was proportional to the EWP concentrations. The WHC of control samples was 75.80% and after addition of 1%, 2% and 3% EWP to the surimi paste, it increased to 84.36%, 88.27% and 92.71% respectively. The addition of different levels of PS into surimi paste following heating at 90°C significantly increased the WHC of the resulting surimi gel compared with the control; however, greater levels of PS were less effective. With SPI, only the lowest level (10%) significantly improved the WHC and increasing the concentration of SPI resulted in no significant difference compared with the control.

These results are similar to those obtained with other fish. In 2005, Tablo-Munizaga and Barbosa-Canovas studied the integrated effect of high pressure and additives such as egg white and potato starch on the physical characteristics of surimi gels from Alaska Pollock and Pacific whiting. They showed that different fish surimi gels responded differently, depending on the nature of the fish proteins. For instance, they reported that the application of 650 MPa without additives on Alaska Pollock surimi gel and 400 MPa with egg white (EW) on Pacific whiting surimi gel resulted in the highest WHC. However, the Alaska Pollock surimi gels treated at 400 MPa without additives and with EW, alone or in combination with PS, showed a significant reduction in WHC compared with heated gels and pressurized gels at 650 MPa. By contrast, when PS was added to Pacific whiting surimi gels, WHC increased significantly, as in the common carp surimi gel studied here.

**The effect of additives on texture properties**

**Puncture test:** The influence of various concentrations of EWP, PS and SPI on breaking force and breaking distance of common carp surimi gel is shown in (Table 3). Increasing the concentration of EWP in common carp surimi increased both breaking force and distance significantly and consequently improved the gel strength of surimi gel. For instance, the recorded gel strength components (breaking force and breaking distance) for the control were 182.73 g and 6.38 mm respectively, whereas after addition of 1% EWP, these values increased to 258.09 g and 7.46 mm respectively. The same trend was observed for the other concentrations of EWP. Surimi with 3% egg white had the most improvement as it enhanced the breaking force and the breaking distance of surimi gel samples by 41% and 31% respectively, by comparison with the control with no added EWP. With PS, the same trend was observed, but the improvement in the resulting surimi gel strength was not proportional to the PS concentration, as surimi gel with the greatest level of PS (12%) showed similar gel strength to the control.

These results are in agreement with Reppond and Babbitt (1993) [22], who reported that the addition of EW up to 2% increased the gel strength of arrow tooth flounder and Alaska Pollock surimi gels. Also, Benjakul and others (2004) [23] indicated that the addition of different levels of egg white (1%, 2% and 3%) into lizardfish surimi increased the breaking force and the breaking distance of the resulting gel samples compared with the control gel with no additive. Moreover, the addition of EW up to 3% increased the gelling properties of lizardfish surimi regardless of heating conditions. Conversely, Tablo-Munizaga and Barbosa-Canovas (2004) reported that the addition of 1% EW in Alaska Pollock and Pacific Whiting surimi paste showed no significant effect on gel strength of the resulting surimi gel, whereas PS (4%) either alone or in combination with EW reduced the gel strength of both surimi gels [24].

In this study, the addition of 10% SPI to the surimi paste resulted in a significant increase in gel strength compared with control samples. The addition of 10% SPI increased the gel strength of Alaska Pollock surimi gel by 36%, 73% and 94% respectively for different additive levels (1%, 2% and 3%). These results are similar to those obtained with other fish [21].

**Table 3:** Puncture test characteristics of common carp surimi with various concentrations of egg white, soy protein isolate and potato starch (mean ± standard deviation).

| Treatment | Breaking force (g) | Breaking distance (mm) | Gel strength |
|-----------|--------------------|------------------------|--------------|
| Con       | 182.73 ± 14.42     | 6.38 ± 0.26            | 1168.81 ± 22.17 |
| EW%1      | 258.09 ± 14.07     | 7.46 ± 0.88            | 1940.44 ± 19.38 |
| EW%2      | 275.62 ± 20.39     | 8.17 ± 0.30            | 2274.08 ± 14.56 |
| EW%3      | 308.22 ± 18.10     | 9.20 ± 0.14            | 2833.62 ± 15.43 |
| PST%3     | 220.72 ± 20.11     | 8.22 ± 0.64            | 1835.19 ± 17.19 |
| PST%6     | 203.63 ± 17.92     | 7.86 ± 0.27            | 1608.14 ± 13.04 |
| PST%12    | 187.45 ± 18.23     | 7.04 ± 0.43            | 1319.48 ± 16.29 |
| SPI%10    | 192.17 ± 13.22     | 6.45 ± 0.70            | 1241.52 ± 18.40 |
| SPI%20    | 164.28 ± 17.60     | 5.82 ± 0.36            | 969.80 ± 17.24 |
| SPI%30    | 142.30 ± 14.19     | 5.43 ± 0.52            | 775.27 ± 13.92 |

Different letters in the same column indicated significant difference (P<0.05) according to a 1-way ANOVA and LSD test.
in a gel with the same gel strength as the control, but increasing the levels of SPI to 20% and 30% significantly decreased the gel strength of the resulting surimi gel. Luo and others (2004) investigated the effects of soy protein isolate (SPI) on the gel properties of different grades of Alaska Pollock and common carp surimi at different setting conditions [7]. Increasing SPI concentrations in direct-cook (85°C for 30 min) and in cook-after-setting (30°C for 60 min) conditions decreased breaking force and distance of gels. These authors stated that SPI was less effective on gel strength of common carp surimi than on Alaska Pollock surimi, as adding 10% SPI increased the breaking force for Alaska Pollock surimi compared with untreated surimi when cooked after incubation at 50°C for 60 min. Furthermore, Luo and others (2006) showed that greater breaking force was obtained by adding 10% SPI in silver carp surimi if cooked after incubation at 50°C for 60 min, compared with direct cooking or heating after setting at 30°C and 40°C for 60 min; moreover, values of breaking force and distance declined when the SPI was increased from 10% to 40% [9]. In addition, Campo and Tavor (2008) studied the effect of starch content on viscoelastic properties of surimi gel and found that “the optimum starch content of Alaska Pollock and common carp surimi at different heating settings [24]. The effect of additives on whiteness of common carp surimi gel

The influence of various concentrations of EWP, SPI and PS on color characteristics of surimi gel samples is shown in Table 4. The addition of EWP significantly improved lightness (L*) compared with the control; however, lightness values for the different levels of added EWP were not significantly different. The improvement in lightness coincided with a decrease in the yellow color (b*value). From the whiteness formula (L*-b*), the treatment with 1% added EWP resulted in the whitest surimi of all the treatments, as the whiteness was improved from 55.21 in the control to 61.82 with 1% EWP. By contrast, according to Park (1994), the addition of 1% dried egg white (DEW) and 1% frozen egg white (FEW) into Alaska Pollock surimi reduced both the lightness and whiteness values of the resulting surimi gel [20]. Also, Benjakul and others (2004) found that EWP addition had no effect on the whiteness of surimi gels prepared under different heating conditions [23]. Hunt et al. [2] reported that adding three types of egg white protein (regular dried egg white (REW), special dried egg white (SEW), and liquid egg white (LEW)) to Alaska Pollock surimi reduced the whiteness of the resulting surimi gels compared with the control sample, apart from 0.5% SEW (P<0.05). With PS, in this study adding only 3% PS to the surimi paste significantly improved either the L* or the b*values of the resulting surimi gel compared with

### Table 4: TPA test characteristics of common carp surimi with various concentrations of egg white, soy protein isolate and potato starch (mean ± standard deviation)

| Treatment type | (N) Hardness | Cohesiveness | (N/s)Adhesiveness | Elasticity |
|----------------|--------------|--------------|-------------------|------------|
| Con            | 27.09 ± 0.44*| 0.64 ± 0.00* | -0.58 ± 0.06*     | 10.19 ± 0.14* |
| EW%1           | 37.19 ± 0.45*| 0.71 ± 0.01* | -0.89 ± 0.02*     | 14.64 ± 0.15* |
| EW%2           | 40.52 ± 0.38*| 0.73 ± 0.00* | -0.98 ± 0.10*     | 15.17 ± 0.43* |
| EW%3           | 44.28 ± 0.22*| 0.75 ± 0.00* | -1.10 ± 0.04*     | 16.10 ± 0.27* |
| PST%3          | 34.14 ± 0.19*| 0.70 ± 0.02* | -0.78 ± 0.06*     | 12.89 ± 0.52* |
| PST%5          | 31.78 ± 0.04*| 0.88 ± 0.01* | -0.73 ± 0.03*     | 11.34 ± 0.72* |
| PST%10         | 28.42 ± 0.62*| 0.76 ± 0.00* | -0.68 ± 0.05*     | 10.58 ± 0.20* |
| PST%20         | 26.19 ± 0.32*| 0.86 ± 0.01* | -0.62 ± 0.03*     | 10.58 ± 0.20* |
| PST%30         | 22.64 ± 0.18*| 0.84 ± 0.00* | -0.59 ± 0.02*     | 10.13 ± 0.62* |
| SPI%3          | 20.36 ± 0.72*| 0.63 ± 0.03* | -0.54 ± 0.02*     | 9.18 ± 0.42*  |
| SPI%10         | 27.33 ± 0.51*| 0.69 ± 0.04* | -0.60 ± 0.03*     | 9.18 ± 0.42*  |
| SPI%20         | 27.33 ± 0.51*| 0.69 ± 0.04* | -0.60 ± 0.03*     | 9.18 ± 0.42*  |

Different letters in the same column indicated significant difference (P<0.05) according to a 1-way ANOVA and LSD test.

**Table 5: Color characteristics of common carp surimi with various concentrations of egg white, soy protein isolate and potato starch (mean ± standard deviation).**
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The sensory characteristics of common carp surimi gel containing different concentrations of EWP, PS and SPI are shown in (Table 6). All three concentrations of EWP significantly affected the color (whiteness) of surimi gel samples but there was no significant difference between treatments with 2% and 3% added EWP. Increasing the EWP concentration reduced the amount of improvement in color. With different concentrations of PS, only the addition of 3% PS showed improved color, whereas the addition of 6% and 12% did not change the color of the surimi gel significantly compared with the control. With SPI, the addition of 20% and 30% SPI decreased the color value of the resulting common carp surimi gel and 10% added SPI had no effect. The panelists selected the surimi sample with 1% added EWP as the best treatment.

EWP at different levels (1%, 2% and 3%) significantly improved the odor of the resulting surimi gel compared with the control. The effects of 1% and 2% added EWP were similar and 3% added EWP was the most effective treatment to improve the odor of surimi gel according to the panelists. With PS, treatments containing 3% and 6% PS scored better for odor than the control, whereas those with 12% PS had no significant difference from the control. Adding 10% SPI into surimi paste did not significantly affect the odor of resulting surimi gel, but increasing its concentration to 20% and 30% reduced the panel scores compared with the control. In flavor the same trend was observed and the 3% added EWP, 3% added PS and 10% added SPI treatments obtained greater scores than the rest. The improvement in the texture of common carp surimi gel blended with EWP was proportional to the different levels of added EWP and the best score for the texture was allocated to the surimi gel with 3% EWP. The same trend was observed in terms of addition of 3% PS and 10% SPI on the texture of the resulting common carp surimi gel. Finally, addition of the three levels (1%, 2% and 3%) of EWP to the common carp surimi paste increased the overall liking of resulting surimi gel by 6.8%, 9.3% and 13.9% respectively, resulting in the panelists giving the greatest score to the treatment with 3% EWP. For PS, treatments containing 3% and 6% PS showed the same results as 3% EWP and the treatment with 12% PS showed no significant difference from the control. Adding only 10% SPI scored the greatest overall liking among different levels of added SPI, as the score for the treatment with 20% SPI was equal to that for the control and the addition of 30% SPI reduced the panel’s overall liking significantly.

Conclusions

Among different the additives tested (EWP, PS and SPI), the addition of EWP at 1%, 2% and 3% concentrations and PS at 3%, 6% and 12% concentrations respectively into surimi paste improved the functional properties of the resulting common carp surimi gel compared with the control. The addition of 3% EWP into the surimi paste caused the greatest viscosity, WHC, gel strength and TPA values of the resulting surimi gel but whiteness decreased due to increasing yellowness. Apart from color, the same trend was observed in treatment with 3% PS. Furthermore, panelists selected the treatments with 3% EWP, 3% PS and 10% SPI as the best treatments to improve the sensory parameters of the resulting surimi gel. The efficacy of other additives such as different types of starch, chitosan etc., either together or individually, at different setting conditions should be investigated to improve the functional properties of common carp surimi.

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Table 6: Sensory evaluation characteristics of common carp surimi with various concentrations of egg white, soy protein isolate and potato starch (mean ± standard deviation).

| Treatment Types | Color (whiteness) | Odor | Flavor | Texture | Overall liking |
|-----------------|-------------------|------|--------|---------|---------------|
| CON             | **7.4 ± 0.8**     | **6.5 ± 0.7** | **6.7 ± 0.9** | **6.8 ± 0.8** | **6.8 ± 1.0** |
| EWP%1           | 7.8 ± 1.0         | 6.9 ± 0.8   | 7.0 ± 1.0 | 7.2 ± 0.9 | 7.3 ± 1.1   |
| EWP%2           | 7.6 ± 0.9         | 6.9 ± 1.1   | 7.2 ± 1.0 | 7.4 ± 1.0 | 7.5 ± 1.0   |
| EWP%3           | **7.6 ± 0.7**     | 7.0 ± 0.8   | 7.1 ± 0.7 | 7.1 ± 0.7 | 7.2 ± 0.3   |
| PST%3           | 7.4 ± 0.9         | 6.9 ± 0.8   | 6.8 ± 0.5 | 6.9 ± 0.9 | 7.0 ± 0.7   |
| PST%6           | 7.3 ± 0.8         | 6.4 ± 0.6   | 6.4 ± 0.8 | 6.7 ± 0.6 | 6.8 ± 0.9   |
| PST%12          | 7.5 ± 0.6         | 6.8 ± 0.7   | 7.1 ± 0.9 | 7.2 ± 1.0 | 7.2 ± 0.8   |
| SPI%10          | 6.6 ± 1.0         | 6.5 ± 0.9   | 6.9 ± 1.0 | 6.8 ± 0.8 | 6.7 ± 0.9   |
| SPI%20          | 6.8 ± 0.8         | 6.2 ± 0.8   | 6.4 ± 0.7 | 6.6 ± 0.7 | 7.0 ± 0.7   |
| SPI%30          | 7.6 ± 0.7         | 6.6 ± 0.5   | 6.7 ± 0.6 | 6.8 ± 0.9 | 6.8 ± 0.7   |

Different letters in the same column indicated significant difference (P<0.05) according to a Mann-Whitney U test.
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