Chicken myofibrillar protein concentrate (CMPC), produced with modified technology used for fish surimi (Dawson et al., 1988; Park et al., 1996), is characterized by very good technological properties, such as a high water retention capacity and a strong ability to form strong gels after being heated. The most frequently used food preservation technique for this kind of product is freezing. Cryoprotectants, such as disaccharides, polysaccharides, polyalcohols, acids and polyphosphates are generally added to surimi to protect myofibrillar proteins from freeze-denaturation during frozen storage and to maintain its high physical and chemical properties.
processability (MacDonald et al., 2005). Measurements of myofibrillar protein solubility SEP (Salt extractable protein), Ca²⁺-ATP-ase activity, unfrozen water by Nuclear Magnet Resonance (NMR), and transition temperatures and enthalpy of myofibrillar proteins by differential scanning calorimetry (DSC) are the most commonly used instrumental methods for the determination of the cryoprotective effects of added substances (Kijowski and Richardson, 1996; Park et al., 1993; Stangierski and Kijowski, 2008; Sych et al., 1990). DSC is an often used as instrumental method for studying thermal behaviour of muscle proteins (Findlay and Barbut, 1990). Color and texture are the major factors responsible for the final acceptability of surimi-like products by consumers. To better suit the textural preferences of consumers, ingredients that modify the textural and water mobility properties of the surimi must be added to surimi paste (Cheow and Yu, 1997). In a composite food such as surimi, additives can modify the texture. Protein additives, such as egg white, serve to increase gel strength and to give a whiter and glossier appearance (Park, 2005). The final surimi-based product can assume almost any desired texture through its gel forming capacity (Park, 1994).

The use of dietary fibre as a cryoprotectant for myofibrillar proteins is not common. Barley bran flour is a dietary fibre that may reduce the risk of heart disease and diabetes (Lupton et al., 1994) and contains a relatively high concentration of β-glucans (7.7%; Bhaty, 1993). Studies have shown that the addition of β-glucans to meat batter increases the denaturation enthalpy of myofibrillar proteins, which suggests that barley bran can interact with meat proteins and stabilize them (Morin et al., 2004).

The objective of this study was to determine the effectiveness of barley bran flour to stabilize chicken myofibrillar proteins during frozen storage.

MATERIAL AND METHODS

Sample preparation
Chicken myofibrillar protein concentrate (CMPC) samples were prepared in the laboratory from mechanically deboned chicken meat (MDCM) using the modified procedure of Yang and Froning (1992). Washing solutions were; distilled water; 0.1 M sodium chloride (NaCl); 0.5% sodium bicarbonate (NaHCO₃); sodium phosphate buffer at pH = 7.2 at an ionic strength of 0.1. In brief, 100 g of MDCM were mixed with 300 mL of washing solutions at 5°C. The meat slurry was mixed with a propeller mixer at 120 rpm for 20 minutes at 5°C. After mixing, the mixture was undisturbed for 5 min, and the fat layer stripped off. The washed meat was collected by centrifugation (800×g, 20 min). The second washing process was the same as the first, and the third washing was carried out using distilled water. Barley bran flour (SiladiAgro d.o.o., Kotoriba, Croatia) was added to the samples in mass fractions of 2, 4 and 6%. Mass fractions were determined as percentage of total mass. The mixture was homogenized in a knife mill Gridomix GM 200 (Retsch, Germany) for 5 minutes. Samples for DSC and color measurements were packed in PE bags and plastic test tubes with an internal diameter of 10 mm for texture analysis. Chicken myofibrillar protein concentrate samples were fast-frozen with liquid nitrogen and stored in a Kirsch ESSENTIAL-280 (Kirsch, Germany) laboratory freezer at –30°C.

Physico-chemical analysis
Textural profile analysis (TPA). Samples of CMPC were placed into plastic test tubes with an internal diameter of 10 mm. After defrosting, the test tubes and their contents were heated for 25 min in a water bath at 80°C. The test tubes containing gels were cooled in ice water until a temperature of approx. 20°C was reached inside the sample. After that, they were stored at 4–6°C until the next day. Texture profile analysis (TPA) tests were performed using a TA.XT2i SMS Stable Micro Systems Texture Analyzer (Stable Microsystems Ltd., Surrey, England) equipped with a cylindrical probe P/75. This involved cutting the samples into 1.5 cm thick slices, which were compressed twice to 60% of their thickness. Force-time curves were recorded at across-head speed of 5 mm s⁻¹ and the recording speed was also 5 mm s⁻¹. The following parameters were quantified (Bourne, 1978): hardness (N), maximum force required to compress the sample, springiness (ratio), the ability of the sample to recover its original form after the deforming force was removed, cohesiveness, the extent to which the sample could be deformed prior to rupture (ratio) and chewiness (N). Chewiness (work required to masticate the
sample before swallowing) is calculated as product of hardness, cohesiveness and springiness.

**Determination of instrumental color.** Color measurements ($L^*$, $a^*$, and $b^*$ values) were taken using a Hunter-Lab Mini ScanXE (A60-1010-615 Model Colorimeter, Hunter-Lab, Reston, VA, USA). The instrument was standardized each time with a black and white ceramic plate ($L^*0 = 93.01, a^*0 = –1.11, and b^*0 = 1.30$). The Hunter $L^*$, $a^*$, and $b^*$ values correspond to lightness, greenness ($–a^*$) or redness ($+a^*$), and blueness ($–b^*$) or yellowness ($+b^*$), respectively. The whiteness ($W_l$) was calculated: $L^* – 3b^*$. The color measurements were performed on CMPC at room temperature ($20 ± 2°C$).

**Thermal analysis.** Differential scanning calorimetry (DSC) was performed using a Mettler Toledo DSC 822\textsuperscript{e} differential scanning calorimeter (Mettler Toledo, Greifensee Switzerland), equipped with STAR\textsuperscript{e} software.

The Mettler Toledo DSC 822\textsuperscript{e} was calibrated with indium ($T_{oi} = 156.6 ±0.3°C, \Delta H_{ti} = 28.45 ±0.6 J g^{-1}$). Samples of ca. 15 mg ($±1 mg$) were weighed and sealed into standard aluminum pans (40 μl), then scanned at temperatures ranging from 25 to 95°C, with a heating rate of 10°C min\textsuperscript{-1}, using an empty standard aluminum pan as a reference. The denaturation (peak) temperatures ($T_p$) were determined using DSC curves. The changes in enthalpy ($\Delta H J g^{-1}$) associated with the denaturation of proteins were determined by measuring the area under the DSC curves using STAR\textsuperscript{e} software. Denaturation enthalpies were expressed as total mass fraction of proteins.

**Statistical analysis.** Denaturation temperatures and denaturation enthalpies ($\Delta H J g^{-1}$) were determined in triplicate, and TPA and color parameters were measured in septuplet for each sample. The experimental data was analyzed using the analysis of variance (ANOVA) and Fisher’s least significant difference (LSD), with significance being defined at $p < 0.05$. Statistical analysis was carried out with Statistica 12.7 (StatSoft Inc. Tulsa, 2015, OK, USA).

**RESULTS AND DISCUSSION**

**Physico-chemical analysis**

**Textural profile analysis (TPA).** The texture profile analysis parameters of CMPC mixed with different mass fractions of barley bran flour ($w = 0–6\%$) during 180 days of frozen storage are shown in Table 1. The sample of CMPC without added barley bran flour showed the lowest values of hardness for all frozen storage time intervals. The hardness of chicken CMPC gels increased significantly ($p < 0.05$) with increasing mass fraction of barley bran at 0, 30, 90 and 180 days of frozen storage. Similarly to hardness, the chewiness of CMPC gels mixed with barley bran flour ($w = 0–6\%$) significantly ($p < 0.05$) increased for all frozen storage time intervals. The springiness of CMPC gels was shown to significantly increase ($p < 0.05$) as the barley bran mass fraction was increased. Frozen storage time decreased the springiness significantly ($p < 0.05$) for all treatments. Similarly to springiness, cohesiveness showed

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**Table 1. Texture profile analysis of CMPC during frozen storage**

<table>
<thead>
<tr>
<th>Barley bran flour %</th>
<th>Days of frozen storage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.05\textsuperscript{A} ±0.11</td>
</tr>
<tr>
<td>4</td>
<td>8.14\textsuperscript{A} ±0.10</td>
</tr>
<tr>
<td>6</td>
<td>9.64\textsuperscript{A} ±0.14</td>
</tr>
</tbody>
</table>

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a significant increase \((p < 0.05)\) with an increased proportion of barley bran flour for all frozen storage intervals. Increasing the length of frozen storage did not significantly \((p > 0.05)\) decrease cohesiveness.

The increase in various texture profile analysis parameters which accompanied the increase in barley bran flour \((w = 0–6\%)\) for all frozen storage intervals are in agreement with the results reported by Alakhrash et al. (2016) for fish surimi with addition of oat bran \((w = 0–8\%)\).

**Instrumental color parameters.** The instrumental color parameters of CMPC with added barley bran flour are presented in Table 2. Generally, the demand is higher for surimi gels with high lightness \((L^*)\), low yellowness \((b^*)\) and high whiteness \((W)\). The color parameters of CMPC were different from the color parameters of fish surimi (Alakhrash et al., 2016; Auh et al., 1999; Tabilo-Munizaga and Barbosa-Canovas, 2004). This could be related to the nature of sample (higher myoglobin content in chicken meat). Similarly, higher values for \(L^*, a^*, b^*\) for pork and chicken surimi in comparison with Alaska Pollock surimi were reported by Jin et al. (2007). The addition of barley bran flour significantly increased \((p < 0.05)\) lightness and whiteness of CMPC samples at all frozen storage intervals. This is in agreement with the studies that investigated the addition of potato starch and egg white and oat bran to Alaska Pollock surimi (Alakhrash et al., 2016; Tabilo-Munizaga and Barbosa-Canovas, 2004). The addition of barley bran flour \((w = 0–6\%)\) to CMPC, in all treatments, for all frozen storage intervals, resulted in decreased \(a^*\) values, indicating a slightly greater green hue in these treatments. The yellowness of the CMPC samples was not significantly affected \((p > 0.05)\) by the addition of barley bran except for the addition of 6% barley bran at 0 and 180 days of frozen storage. Frozen storage did not significantly affect \((p < 0.05)\) instrumental color parameters of CMPC \((L^*, a^*, b^*\) and \(W); Table 2).
mixed with barley bran flour. Referring to previous DSC studies of similar samples (Aktas et al., 2005; Barbut and Findlay, 1991; Kovačević and Mastanjević, 2011; Sych et al., 1991), it can be assumed that two peaks in this study are related to the thermal denaturation of myosin and actin. Denaturation temperatures ($T_d$) of myosin and actin for CMPC samples mixed with barley bran flour ($w = 0–6\%$) at 0, 30, 90 and 180 days of frozen storage are presented in Table 3. The denaturation temperatures ($T_d$) of myosin and actin were different from the denaturation temperatures of chicken meat reported by Barbut and Findlay (1991) and Bircan and Barringer (2002). Yang and Froning (1994) and Kijowski and Richardson (1996) reported similar results for washed mechanically-deboned poultry meat. This could be related to by concentration of myofibrillar proteins by washing, and the different pH and ionic environment when compared to the raw state of muscle (Lesiow and Xiong, 2001). Myosin’s $T_d$ varied significantly ($p < 0.05$) as a function of mass fraction of barley bran flour, but not as a function of frozen storage time (Table 1). These increases in $T_d$ of myosin as the mass fraction of barley bran flour was increased could be interpreted as the stabilization of myofibrillar proteins, since a higher temperature was required to denature these proteins. $T_d$ of actin denaturation varied significantly ($p < 0.05$) with the addition of barley bran flour, but not with frozen storage time (Table 2). The denaturation enthalpies of myosin and actin of CMPC samples with addition of barley bran flour.
flour, during 180 days of frozen storage, are shown in Figure 1 and 2. The values of ΔH of myosin and actin decreased with increasing of storage time (Herrera et al., 2001; Stangierski and Kijowski, 2008; Kovačević and Mastanjević, 2014). The largest decreases in ΔH of myosin and actin in all samples were in the first 30

<table>
<thead>
<tr>
<th>Barley bran flour</th>
<th>Days of frozen storage</th>
<th>Myosin</th>
<th>Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ΔH</td>
<td>ΔH</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>60.97ab ±0.19</td>
<td>72.71ab ±0.19</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>60.92bc ±0.21</td>
<td>71.28bc ±0.21</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>61.30bc ±0.30</td>
<td>72.86bc ±0.30</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>60.37bc ±0.10</td>
<td>72.28bc ±0.10</td>
</tr>
<tr>
<td>2</td>
<td>64.60bc ±0.10</td>
<td>64.90bc ±0.08</td>
<td>73.44bc ±0.08</td>
</tr>
<tr>
<td>4</td>
<td>65.03bc ±0.11</td>
<td>61.38bc ±0.07</td>
<td>73.41bc ±0.07</td>
</tr>
<tr>
<td>6</td>
<td>65.49bc ±0.04</td>
<td>61.58bc ±0.29</td>
<td>74.09bc ±0.29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>64.54ab ±0.24</td>
<td>74.08bc ±0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>65.81ab ±0.11</td>
<td>73.79bc ±0.11</td>
</tr>
</tbody>
</table>

Values are means ±SD of triplicate.
Values in the same column with different superscripts a–d or in same row (A–C) are significantly different (p < 0.05).

**Table 3.** Temperatures of denaturation of CMPC myosin and actin during frozen storage

**Fig. 1.** Changes in denaturation enthalpy of the myosin of CMPC samples as a function of mass fraction of barley bran flour and frozen storage time
days of frozen storage, especially for samples without additives (Fig. 1). The values of $\Delta H$ for myosin and actin were shown to increase when the mass fraction of barley bran flour was increased, with the exception of day 0. The $\Delta H$ of myosin varied significantly ($p < 0.05$) as a function of mass fraction of barley bran flour and as a function of frozen storage time (Fig. 1). For actin, $\Delta H$ varied significantly ($p < 0.05$) as a function of mass fraction of barley bran and as a function of frozen storage time (Fig. 2).

**CONCLUSIONS**

The denaturation temperatures and enthalpies and some instrumental color and texture parameters of chicken myofibrillar proteins were shown to increase when the mass fraction of barley bran flour was increased, for all frozen storage time intervals. Since these values are directly related to the amount of native proteins, higher values of denaturation enthalpies indicate possible interactions and the stabilization of chicken myofibrillar proteins with the addition of barley bran flour.

**REFERENCES**


