



# Changes in properties of food proteins after interaction with free and $\beta$ -cyclodextrin encapsulated hydroxycinnamic acids

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**Abstract** Polyphenol–protein interactions during food processing may cause deterioration in protein nutritional quality status. This limits the possibility to enrich foods in phenolic preparations. The aim of this study was to assess whether encapsulation of hydroxycinnamic acids (HCAs) from green coffee extract (GCE) [using inclusion in  $\beta$ -cyclodextrin ( $\beta$ -CD)] limits polyphenol–protein interactions. Three different protein isolates: egg white proteins, whey protein concentrate and soy protein isolate were exposed to interact with HCAs from GCE. After interactions, a higher increase in molecular mass and higher degradation of essential amino acids, as well as limitation of proteolytic digestion and solubility of proteins were reported, compared with the controls processed without HCAs. On the other hand, the interactions contributed to a higher radical-scavenging capacity in DPPH $\cdot$  and OH $\cdot$  assays and color deepening of proteins during processing. Inclusion of HCAs in  $\beta$ -CD cavity resulted in the limitation of the protein–HCA interactions, thus decreased the changes in nutritional and physicochemical properties. Adding HCAs in the form of inclusion complexes could be a promising solution for reducing unfavorable protein–polyphenol interactions during processing of foods enriched with phenolic acids.

**Keywords** Green coffee · Hydroxycinnamic acids ·  $\beta$ -Cyclodextrin · Protein–polyphenol interactions · LC–MS<sup>n</sup>

## Introduction

Modern food should have high nutritional value, attractive sensory characteristics and health-promoting properties. Therefore, it is not surprising that this type of food is often enriched with protein preparations that allow achieving high nutritional value and proper texture characteristics [1]. Furthermore, functional food is also often enriched with natural antioxidants, which have to protect ingredients from oxidation and additionally cause pro-healthy effects in human organism [2]. However, the combination of proteins and polyphenols in one product may cause various chemical interactions. As a consequence of those interactions, bioavailability or health-promoting properties of proteins and antioxidants can be reduced.

According to numerous studies hydroxycinnamic acids (HCAs) have multitude of health benefits. HCAs such as caffeic and ferulic acids, and their esters with quinic acid are one of the most important groups of phenolic compounds [3]. They have been shown to exhibit a broad range of biological activities including: antibacterial, antifungal, hepatoprotective, antithrombotic, anti-inflammatory, hypoglycemic and antioxidant [4–10]. In vivo antioxidant activity of HCAs reduces the risk of several oxidative stress-related diseases, including atherosclerosis, some kinds of cancer and Alzheimer's disease [11–13]. Coffee is one of the plants that accumulate HCAs in quantities sufficient to have physiological effects. Green coffee beans are rich source of HCAs. They contain 4–10 % of these compounds [14]. Coffee bean extracts could be added to various kinds of food products, including those rich in proteins [15]. Previous studies have shown that this kind of combinations caused partial degradation of essential amino acids and reduced the susceptibility to proteolytic digestion [16, 17]. Therefore, it could be more beneficial to encapsulate

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HCAs by forming inclusion complexes with  $\beta$ -cyclodextrin ( $\beta$ -CD) and to add them in this form to foods [18–20]. The aromatic ring of HCA located by inclusion inside the  $\beta$ -CD cavity can be protected against interactions. So far the limitation of interactions of polyphenol oxidase with inclusion complex of 5-caffeoylquinic acid (5-CQA) with  $\beta$ -CD was confirmed [21]. Inclusion of HCAs with  $\beta$ -CD presumably does not limit their bioavailability and antioxidant activity [22].

The authors demonstrated lesser extent of food protein interactions with hydroxycinnamic acids from coffee encapsulated in  $\beta$ -CD compared with free hydroxycinnamic acids that caused higher availability of the polyphenols for the digestive tract [23]. The aim of this study was to determine the extent to which encapsulation of HCAs from green coffee beans by inclusion in  $\beta$ -CD may limit the deterioration of food protein properties, caused by protein–HCA interactions.

## Materials and methods

### Chemicals and raw materials

Analytical-grade ethanol, ethyl acetate and 30 % hydrogen peroxide were purchased from Poch (Gliwice, Poland), hydrochloric acid, sodium chloride, copper sulfate pentahydrate, potassium sodium tartrate tetrahydrate, potassium iodide, calcium carbonate, urea, sodium metabisulfite and sodium hydroxide from Chempur (Piekary Śląskie, Poland). HPLC-grade methanol, bovine serum albumin (~99 %), phenol,  $\beta$ -cyclodextrin ( $\beta$ -CD,  $\geq 98$  %) and 2,2-diphenyl-1-picrylhydrazyl (DPPH $\cdot$ ) were purchased from Fluka (St. Louis, MO, USA), 5-caffeoylquinic acid (5-CQA, ~99 %), caffeic acid (CA, ~99 %), ferulic acid (FA, ~99 %), pepsin (~99 %), trypsin (~99 %), chymotrypsin (~99 %), sodium phosphate monobasic dihydrate (~99 %), sodium phosphate dibasic dodecahydrate (~99 %), iron (II) sulfate (~98 %), sodium salicylate (~98 %) and tris-hydrochloric acid (~98 %) from Sigma-Aldrich (St. Louis, MO, USA), acetate buffer pH 2.20, phosphate buffer pH 6.45 and set of reagents for amino acids analysis from BioChrom (Cambridge, UK), and nylon filters from Chromacol (Herts, UK). Ultrapure water (resistivity, 18.2 M $\Omega$  cm) was obtained from a Millipore Milli-Q Plus purification system (Bedford, MA, USA).

Green robusta coffee beans (*Coffea canephora* L.) harvested in Brazil in 2012, dehulled by dry method were purchased from Bero Polska (Gdynia, Poland). Protein isolates: whey protein concentrate (WPC, protein content (pc) 873 g kg $^{-1}$ , lactose 56 g kg $^{-1}$ , ash 22 g kg $^{-1}$ ) from Bartex (Paślęk, Poland), egg white proteins (EWP, pc 892 g kg $^{-1}$ , fat 1 g kg $^{-1}$ , ash 51 g kg $^{-1}$ ) from Basso (Gołkowiec,

Poland) and soy protein isolate (SPI, pc 905 g kg $^{-1}$ , fiber 2 g kg $^{-1}$ , ash 40 g kg $^{-1}$ ) from Gushen (Pabianice, Poland) were used for interactions with HCAs.

### Preparation and purification of green coffee extract (GCE)

The aqueous extract was obtained as previously described [14]. The extract contained isomers of caffeoylquinic, feruloylquinic and dicaffeoylquinic acids. The solution was frozen at  $-80$  °C, freeze-dried in a DELTA 1-24LSC Christ freeze drier (Osterode am Harz, Germany) and purified. To purify GCE, the centrifugal partition chromatography (CPC) method was used with SPOT Prep II 50 chromatograph from Armen Instrument (Saint-Avé, France) integrated with UV/Vis detector and a fraction collector. Briefly, the two-phase system of solvents was prepared from water, ethanol and ethyl acetate (5:1:4, v/v/v). Elution of HCAs occurred from 20 to 24 min and from 29 to 32 min of the analysis, detected by UV absorption at 320 nm. Collected fractions were concentrated in ScanMaxiVac Labogene (Lyngø, Denmark) concentrator and again freeze-dried. The content of HCAs in purified GCE was 564.8 g kg $^{-1}$  db. (dry basis) and caffeine was eliminated. The remainder of the extract was proteins (29.5 g kg $^{-1}$  db.), sugars (92.8 g kg $^{-1}$  db.), soluble fiber (121.6 g kg $^{-1}$  db.) and minerals (191.3 g kg $^{-1}$  db.).

### Preparation of inclusion complexes of $\beta$ -CD with HCAs from GCE ( $\beta$ -CD–HCAs)

Inclusion complexes of  $\beta$ -CD with HCAs were prepared with average molar ratio of substrates 1:2 (calculated based on molar mass of 5-CQA) in the reaction solution of substrates to maximize the efficacy of complexation [14]. Therefore, 0.1135 g of  $\beta$ -CD and 0.1303 g of GCE, which contained 0.0708 g of HCAs were dissolved in 2 mL of water. The complexation was conducted for 2 h at 50 °C in a Pierce Reacti-Therm TS-18821 reactor from Thermo Scientific (Palo Alto, CA, USA). After complexation, the solution was left for 24 h at 0 °C and the suspension was centrifuged in MIKRO 22R centrifuge from Hettich (Kirchlengern, Germany) at 4 °C for 20 min at 10,000 $\times g$ . A mixture of  $\beta$ -CD complexes with particular HCAs was obtained and characterized by ESI–MS/MS method [14]. The molar ratio of  $\beta$ -CD and HCAs in complexes was 1:1.

### Interactions of HCAs with proteins

A 0.10-g sample of WPC, EWP or SPI and 0.015 g of HCAs contained in 0.028 g of GCE or in 0.075 g of  $\beta$ -CD–HCA complexes were dissolved in 3 mL of phosphate buffer pH 6.45. Proteins (0.10 g) were additionally reacted with 0.01 g of standards: 5-CQA, CA or FA. The solutions

were reacted for 1.5 h at 25 or 90 °C in a Pierce Reacti-Therm TS-18821 reactor and dialyzed in micro dialyzer against cellulose membrane from Harvard Apparatus (Holliston, MA, US) to remove molecules  $\leq 1.5$  kDa and then freeze-dried [23].

#### Molecular mass analysis

A 5-mg sample of protein was dissolved in 1 mL of phosphate buffer (pH 6.8) obtained by dissolving monobasic sodium phosphate dihydrate and dibasic sodium phosphate dodecahydrate. HPLC analysis was conducted at 25 °C with isocratic elution using phosphate buffer pH 6.8 [24]. Liquid chromatograph (UHPLC) UltiMate 3000 Dionex (Sunnyvale, CA, USA), equipped with UV/DAD detector and gel column Yarra SEC-3000 (3,000  $\times$  7.8 mm, 3  $\mu$ m) from Phenomenex (Torrance, CA, USA) was used. Detection was performed at 280 nm. The injection volume was 10  $\mu$ L, and the flow rate was 1.0 mL min<sup>-1</sup>. To calculate the molecular mass, we used standard mixture of proteins. The mixture contained bovine thyroglobulin (670 kDa), globulin [IgA (300 kDa) + IgG (150 kDa)], ovalbumin (44 kDa), myoglobin (17 kDa) and uridine (244 Da), purchased from Phenomenex (Torrance, CA, USA). Molecular masses were calculated based on the chromatogram of standard solution. For this purpose, an equation was obtained based on logarithmic curve of the molecular mass of each standard component related to its individual elution time divided by the elution time of the first component (thyroglobulin). The analysis was also performed after proteolytic digestion.

#### Essential amino acids profile determination

The analysis was performed according to the Commission Directive 98/64/EC [25]. Oxidative solution in volume of 0.5 mL (0.05 mL of 30 % hydrogen peroxide with 0.45 mL of formic acid solution (8.89 g of formic acid, 0.47 g of phenol and 11.1 g of water)) was added to 10 mg of proteins. Labile amino acids such as methionine and cysteine were fully oxidized to methionine sulfone and L-cysteic acid, respectively. Then, 0.084 g of sodium metabisulfite was added to remove an excess of oxidizing agent. Subsequently, 2.5 mL of hydrolysis solution (49.2 mL of 37 % hydrochloric acid, 1 g of phenol and water to 100 ml) was added and kept at 104 °C for 24 h. Next, 1 mL of 40 % sodium hydroxide was added followed by acetate buffer pH 2.20 to a volume of 5 mL. An aliquot of the resulting solution was diluted five times with acetate buffer pH 2.2 and filtered through a 0.2- $\mu$ m nylon filter. Chromatographic analysis was carried out using an amino acid analyzer Biochrom 30+ with oxidized feedstuff column, with post-column ninhydrin detection. Volume of injection

was 20  $\mu$ L. Amino acids elution using set of buffers was as follows: at 56 °C: 5 min at pH 2.65 and 19.5 min at pH 3.35, then at 58 °C: 21.5 min at pH 4.25 and 1 min at pH 8.6, next at 68 °C: 18 min at pH 8.6 and finally at 94 °C: 25 min at pH 8.6 and 4 min with 20 % NaOH and ninhydrin. The flow rate during the whole analysis was 0.4 mL min<sup>-1</sup>.

#### Proteolytic digestibility analysis

Proteolytic digestion was performed according to Rawel et al. [24]. A 6-mg sample of protein was dissolved in 1.5 mL of pH 2.0 buffer (0.3 mL of 37 % hydrochloric acid, 12 mL of 1 M urea and water to 100 mL) and 100  $\mu$ L of pepsin (1 mg mL<sup>-1</sup>) was added. Samples were incubated for 2 h at 36 °C. After this step, 0.27 mL of 5 % sodium hydroxide was added. The samples were frozen and then freeze-dried. Subsequently, 1.5 mL of pH 8.2 buffer (0.02 M calcium carbonate, 2 M urea, 0.1 M tris-hydrochloric acid pH 8) together with 100  $\mu$ L (1 mg mL<sup>-1</sup>) of both trypsin and chymotrypsin were added. Prepared samples were re-incubated for 24 h at 36 °C and then frozen and freeze-dried.

#### Radical-scavenging capacity

Scavenging capacity against DPPH<sup>·</sup> radical was determined according to MacDonald-Wicks et al. [26] with some modifications. The test was carried out using protein solutions in phosphate buffer pH 6.8 at concentrations of 10, 25, 50 and 100 mg mL<sup>-1</sup>. Next, 0.1 mL of protein solution was reacted with 3.9 mL of methanolic radical solution (50  $\mu$ g mL<sup>-1</sup>). Methanol was used as a blank sample and 0.1 mL of pH 6.8 buffer with 3.9 mL of methanolic DPPH<sup>·</sup> solution as a control. Based on the measurement of absorbance at 517 nm of a tested sample ( $A_{\text{test}}$ ) and control ( $A_{\text{control}}$ ) after 30 min [UV/Vis spectrophotometer U-2800 A Hitachi (Tokyo, Japan)], a calibration curve of protein concentration versus DPPH<sup>·</sup> radical-scavenging capacity and its regression equation were obtained. DPPH<sup>·</sup> radical-scavenging capacity AA % =  $[(A_{\text{control}} - A_{\text{test}})/A_{\text{control}}] \times 100$  %.

Hydroxyl radical-scavenging capacity of protein solutions in phosphate buffer pH 6.8 was performed according to Sudha et al. [27]. Briefly 1 mL of 1.5 mM iron (II) sulfate was combined with 0.7 mL of 6 mM hydrogen peroxide and 0.3 mL of 20 mM sodium salicylate. Samples were incubated for 1 h at 37 °C, and the absorbance of hydroxylated salicylate complex was measured at 562 nm. OH<sup>·</sup> radical-scavenging capacity AA % =  $[1 - (A_1 - A_2)/A_0] \times 100$ , where  $A_0$  is the absorbance of the control (without proteins),  $A_1$ —in the presence of protein, and  $A_2$ —without sodium salicylate.

### CIE $L^*a^*b^*$ color determination

Color measurements in CIE  $L^*a^*b^*$  system were performed using an automatic colorimeter Konica Minolta CR-400 with Spectra Magic NX 1.3 software (Osaka, Japan). Color parameters, i.e.,  $L^*$  (from 0—black to 100—white),  $a^*$  [from (−50)—green to 50—red] and  $b^*$  [from (−50)—blue to 50—yellow] were measured [28].

### Protein solubility

The solubility of proteins was determined by the biuret method by dissolving in 0.9 % sodium chloride ( $1 \text{ mg mL}^{-1}$ ) [24]. To 1 mL of the protein solution, 4 mL of biuret reagent (1.5 g of copper sulfate, 6.9 g of potassium sodium tartrate, 300 ml of 10 % sodium hydroxide and 2 g of potassium iodide filled up to 1 L with water) was added. Absorbance was measured after 30 min at 540 nm. As a reference, a sample without protein was prepared. A calibration curve was obtained using bovine serum albumin dissolved in 0.9 % sodium chloride.

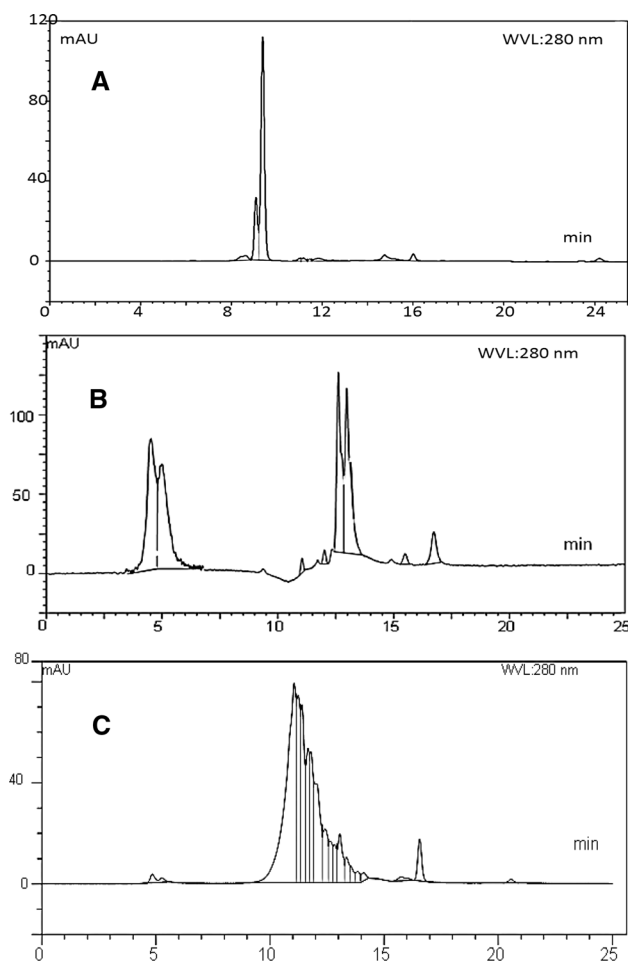
### Statistical analysis

The protein–HCA interactions were conducted twice. Analyses were carried out in triplicate for both replicates, and their results were subjected to statistical analysis. It comprised determination of average values of six measurements and their standard deviation as well as one-way ANOVA (analysis of variation) using Statistica 10.0 software at the significance level  $p < 0.05$ .

## Results and discussion

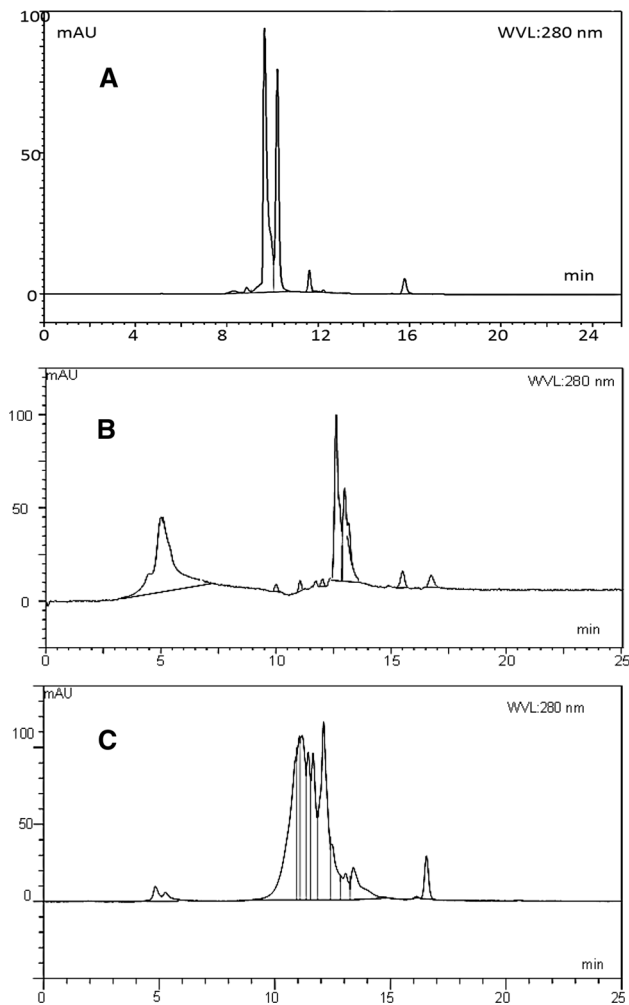
### Molecular mass and proteolytic digestion susceptibility of proteins after interactions with HCAs

Three different commercial protein isolates were interacting with HCAs from green coffee beans. HCAs were used in a form of purified GCE and  $\beta$ -CD–HCAs. The interactions were additionally carried out with standards of HCAs, i.e., CA, 5-CQA and FA. Report on molecular mass changes in proteins as a result of interactions with HCAs was obtained with the use of HPLC–UV/DAD method. Examples of obtained chromatograms are presented in Figs. 1, 2 and 3a, b, wherein (a) represents the chromatograms of unmodified EWP, WPC and SPI and (b) after heating with HCAs from GCE at pH 6.45 and 90 °C. Average molecular mass (AMM, Table 1) was calculated from molecular mass corresponding to the recorded peaks and their share in the total peak area. Unmodified proteins showed AMM of 50, 29 and 191 kDa, respectively, for



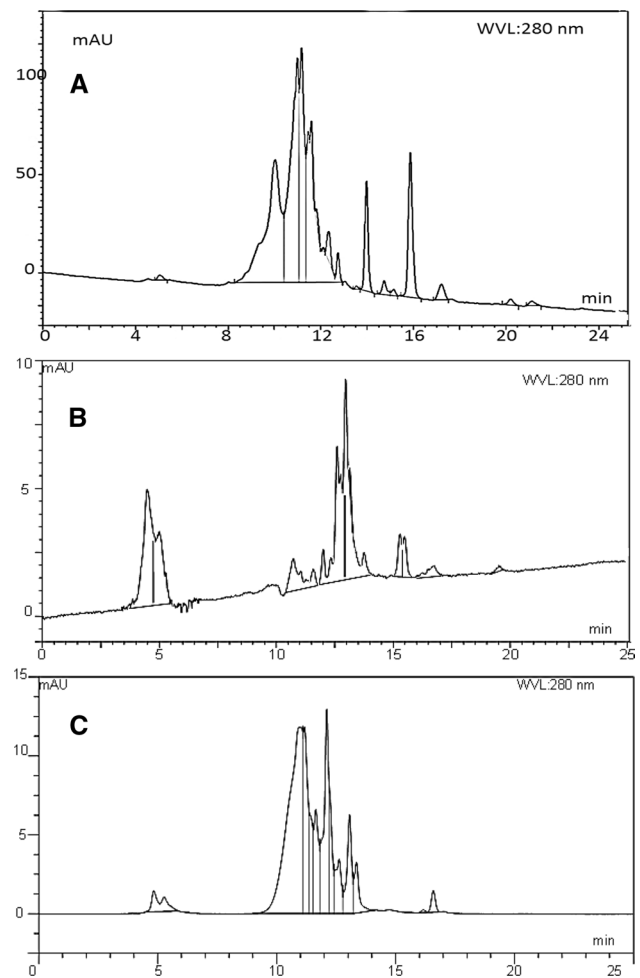
**Fig. 1** HPLC gel-filtration chromatogram of egg white proteins **a** unmodified, **b** interacted at pH 6.45 and 90 °C with hydroxycinnamic acids from green coffee extract and **c** interacting as previously and proteolysed

EWP, WPC and SPI. Proteins that were modified without heating showed AMM increase of maximum 376 % (from 191.22 to 910.63 kDa; SPI + GCE, 25 °C) (Table 1). For proteins exposed to temperature of 90 °C without HCAs AMM increase of 342, 386 and 677 % for EWP (from 49.76 to 220.13 kDa), SPI (from 191.22 to 930.25 kDa) and WPC (from 28.87 to 224.34 kDa) was observed, respectively. However, after heating of proteins together with HCAs, a much greater AMM growth of 465 % (from 191.22 to 1,080.36 kDa for SPI +  $\beta$ -CD–HCAs) to 3,974 % (from 49.76 to 1,937.82 for EWP + 5-CQA) was reported. The large products of interactions were formed as a result of aggregation or cross-linking as it was shown by Faergemant et al. [29] and Rawel et al. [30]. From the above, it could be concluded that higher temperature promoted the intensity of interactions between the proteins and polyphenols carried out at pH 6.45 [17]. HCAs inclusion in the  $\beta$ -CD molecule resulted in limitation of AMM increase



**Fig. 2** HPLC gel-filtration chromatogram of whey proteins concentrate **a** unmodified, **b** interacted at pH 6.45 and 90 °C with hydroxycinnamic acids from green coffee extract and **c** interacting as previously and proteolysed

compared with proteins heated with free HCAs. For this option, AMM was on the similar level as for proteins heated without HCAs and statistically lower ( $p < 0.05$ ) than for proteins heated with free HCAs. The increase in protein AMM for different kinds of isolates followed in order: SPI < WPC < EWP, so in opposite order than the initial AMM. Such relation was caused by the fact that initial high value of molecular mass (for SPI) resulted in relatively small protein surface, on which the interactions take place [23]. Different forms of HCAs preparations showed their impact on AMM increase in the following order:  $\beta$ -CD-HCAs < FA < CA < GCE < 5-CQA. Therefore, methoxylation of one of the hydroxyl groups on FA phenolic ring compared with CA limited interactions with proteins. On the contrary, quinic acid moiety in 5-CQA intensified interactions related to the AMM increase. Similar observation was made by Rawel et al. [24].



**Fig. 3** HPLC gel-filtration chromatogram of soy protein isolate **a** unmodified, **b** interacted at pH 6.45 and 90 °C with hydroxycinnamic acids from green coffee extract and **c** interacting as previously and proteolysed

The profile of molecular mass of each digested protein was analyzed by HPLC–UV/DAD method and examples of chromatograms are presented in Figs. 1, 2 and 3c. Aggregation or cross-linking affected digestion of proteins. For proteins after digestion, the lowest AMM was reported for unmodified EWP, SPI and WPC and it amounted about 5, 4, and 6.5 kDa, respectively (Table 1). Processing of proteins without HCAs contributed to AMM increase after digestion of 7–99 % (from 6.09 to 6.51 kDa for SPI at 25 °C and from 3.14 to 6.26 kDa for WPC at 90 °C, respectively). When the process was carried with HCAs at ambient temperature, AMM for digested SPI was statistically at the same level ( $p < 0.05$ ), (decreased from 6.09 to 5.87 kDa) and for EWP and WPC increased by 80 % (from 3.19 to 5.76 kDa and from 3.14 to 5.65 kDa, respectively). This indicates that agglomerated and cross-linked protein molecules are less accessible for enzymes [30]. Additionally, binding of HCAs to hydrophobic amino acids that are cleaved during

**Table 1** Average molecular mass (AMM) (kDa) of proteins: egg white proteins (EWP), whey protein concentrate (WPC) and soy protein isolate (SPI) interacted at pH 6.45 and 25 or 90 °C with hydroxycinnamic acids (HCAs): from green coffee extract (GCE) or as  $\beta$ -cyclodextrin inclusion complexes ( $\beta$ -CD-HCAs) or standards of caffeic acid (CA), 5-caffeoylquinic acid (5-CQA) and ferulic acid (FA);  $n = 6$ ,  $\pm$ SD, different letters in one row correspond to significant differences ( $p < 0.05$ )

HCAs preparation and interaction temperature (°C)									
Unmodified	Control 25	Control 90	GCE 25	GCE 90	$\beta$ -CD-HCAs 90	CA 90	5-CQA 90	FA 90	
EWP									
49.76 $\pm$ 2.55 <sup>a</sup>	50.53 $\pm$ 3.07 <sup>a</sup>	220.13 $\pm$ 9.15	91.14 $\pm$ 5.46	1,684.45 $\pm$ 63.72	301.76 $\pm$ 11.28	1,477.76 $\pm$ 47.46	1,937.82 $\pm$ 83.22	1,276.82 $\pm$ 51.18	
EWP after proteolytic digestion									
3.19 $\pm$ 0.23	5.02 $\pm$ 0.37	5.75 $\pm$ 0.17	5.76 $\pm$ 0.42 <sup>a</sup>	6.29 $\pm$ 0.30 <sup>ab</sup>	5.83 $\pm$ 0.27 <sup>a</sup>	6.70 $\pm$ 0.43 <sup>b</sup>	6.24 $\pm$ 0.29 <sup>ab</sup>	6.02 $\pm$ 0.12 <sup>a</sup>	
WPC									
28.87 $\pm$ 1.47	69.06 $\pm$ 2.96	224.34 $\pm$ 7.73	133.50 $\pm$ 5.06 <sup>a</sup>	486.54 $\pm$ 11.38	208.84 $\pm$ 7.12	285.59 $\pm$ 8.75	444.93 $\pm$ 12.63	140.08 $\pm$ 4.21 <sup>a</sup>	
WPC after proteolytic digestion									
3.14 $\pm$ 0.34	4.37 $\pm$ 0.31 <sup>a</sup>	6.26 $\pm$ 0.57 <sup>d</sup>	5.65 $\pm$ 0.65 <sup>c,d</sup>	5.88 $\pm$ 0.40 <sup>c,d</sup>	4.75 $\pm$ 0.18 <sup>ab</sup>	5.46 $\pm$ 0.26 <sup>c</sup>	5.25 $\pm$ 0.43 <sup>b,c</sup>	4.77 $\pm$ 0.37 <sup>ab</sup>	
SPI									
191.22 $\pm$ 6.20	435.49 $\pm$ 9.17	910.63 $\pm$ 15.67 <sup>a</sup>	930.25 $\pm$ 23.18 <sup>a</sup>	1,430.95 $\pm$ 37.12	1,080.36 $\pm$ 30.28 <sup>b</sup>	1,226.10 $\pm$ 38.60	1,348.12 $\pm$ 48.41	1,113.49 $\pm$ 26.87 <sup>b</sup>	
SPI after proteolytic digestion									
6.09 $\pm$ 0.43 <sup>b,c</sup>	6.51 $\pm$ 0.29 <sup>c</sup>	4.73 $\pm$ 0.25 <sup>a</sup>	5.87 $\pm$ 0.31 <sup>b</sup>	13.88 $\pm$ 0.47	5.04 $\pm$ 0.09 <sup>a</sup>	7.98 $\pm$ 0.17	6.23 $\pm$ 0.62 <sup>b,c</sup>	8.63 $\pm$ 0.28	

digestion by pepsin, limits availability of nearest peptide bonds for enzyme [31]. Furthermore, proteolytic enzymes themselves could interact with HCAs, what decreases their activity [32, 33]. For SPI, greater efficacy of proteolytic digestion may be due to conformational changes caused by interactions with HCAs and exposure of hydrophobic amino acids [34]. Heating of proteins with HCAs contributed to changes in AMM of digested proteins in the range from  $-17\%$  (from 6.09 to 5.04 kDa) for SPI +  $\beta$ -CD-HCAs to  $128\%$  (from 6.09 to 13.88 kDa) for SPI + GCE. Taking into consideration protein type, AMM after digestion increased in the following order: SPI < WPC < EWP, while in the relation to HCAs form, the increase in AMM was as follows:  $\beta$ -CD-HCAs < FA < 5-CQA < CA < GCE. Alike for result of this study greater limitation of the proteolytic digestion by CA compared to 5-CQA observed Rawel et al. [35].

#### Amino acids profile of proteins after interactions with HCAs

The profile of essential amino acids was analyzed after hydrolysis with hydrochloric acid. Total amino acids were also determined to give insight on the overall amino acids changes. In unmodified proteins, the content of essential as well as total amino acids increased in the following order: SPI < EWP < WPC (Table 2). The analysis showed losses of the essential and total amino acids as a result of processing. These losses were increased in the presence of HCAs during modification due to stable HCAs binding to amino, thiol and phenolic moieties [36]. The used protocol of amino acids determination caused complete degradation of tryptophan, which was not reported in the mixture of amino acids after hydrolysis. However, the interactions of tryptophan with HCAs caused its protection during hydrolysis, and hence some amounts were determined in the proteins processed with HCAs. In general, the greatest losses were observed for methionine, followed by valine and isoleucine. The influence of HCAs preparation form on essential amino acids losses increased in order:  $\beta$ -CD-HCAs < 5-CQA < FA < CA < GCE. Therefore, inclusion of HCAs in the  $\beta$ -CD had a beneficial effect on the retention of essential and total amino acids during processing. Comparing the three analyzed proteins, average loss of essential amino acids increased in order: SPI < EWP < WPC, so in the case of the analyzed isolates, the greater the initial content of essential amino acids was, the bigger the losses during processing.

#### Radical-scavenging capacity of proteins after interactions with HCAs

Products of EWP, SPI and WPC interactions with HCAs were analyzed on DPPH $\cdot$  and OH $\cdot$  radicals scavenging capacity.

**Table 2** Essential and total amino acids concentration in proteins (g kg<sup>-1</sup> db.): whey protein concentrate (WPC), egg white proteins (EWP) and soy protein isolate (SPI) interacted at pH 6.45 and 25 or 90 °C with hydroxycinnamic acids (HCAs): from green coffee extract (GCE) or as β-cyclodextrin inclusion complexes (β-CD–HCAs) or standards of caffeic acid (CA), 5-caffeoylquinic acid (5-CQA) and ferulic acid (FA); n = 6, ND—not detected; ±SD, different letters in one row correspond to significant differences (p < 0.05)

HCAs preparation and interaction temperature (°C)										
Amino acid	Unmodified	Control 25	Control 90	GCE 25	GCE 90	β-CD–HCAs 90	CA 90	5-CQA 90	FA 90	
<i>WPC</i>										
Methionine	26.4 ± 1.2	21.5 ± 0.9 <sup>d</sup>	20.5 ± 1.1 <sup>d</sup>	20.8 ± 1.5 <sup>d</sup>	12.4 ± 0.6 <sup>b</sup>	17.6 ± 1.0 <sup>c</sup>	16.9 ± 1.3 <sup>c</sup>	9.4 ± 0.5 <sup>a</sup>	11.0 ± 0.8 <sup>ab</sup>	
Threonine	71.2 ± 4.3 <sup>f</sup>	67.4 ± 3.3 <sup>ef</sup>	57.0 ± 3.8 <sup>d</sup>	60.6 ± 2.1 <sup>d</sup>	51.5 ± 4.5 <sup>bc</sup>	67.4 ± 3.9 <sup>ef</sup>	42.9 ± 2.2 <sup>a</sup>	45.8 ± 3.6 <sup>ab</sup>	52.0 ± 1.9 <sup>c</sup>	
Valine	68.6 ± 2.5	50.8 ± 1.4 <sup>c</sup>	39.0 ± 1.4 <sup>ab</sup>	48.5 ± 2.0 <sup>c</sup>	38.5 ± 2.2 <sup>ab</sup>	55.0 ± 3.1	36.6 ± 2.9 <sup>a</sup>	36.9 ± 1.8 <sup>a</sup>	43.4 ± 2.7 <sup>b</sup>	
Isoleucine	79.2 ± 3.8	61.9 ± 4.4 <sup>b</sup>	45.3 ± 2.7 <sup>a</sup>	61.2 ± 2.1 <sup>b</sup>	42.8 ± 1.9 <sup>a</sup>	69.5 ± 4.1	45.7 ± 2.2 <sup>a</sup>	36.4 ± 2.8	53.3 ± 3.0	
Leucine	124.0 ± 7.6 <sup>d</sup>	120.4 ± 4.3 <sup>d</sup>	103.5 ± 6.8 <sup>bc</sup>	109.2 ± 5.5	103.2 ± 3.6 <sup>bc</sup>	124.0 ± 7.1 <sup>d</sup>	86.7 ± 6.5 <sup>a</sup>	98.2 ± 5.4 <sup>ab</sup>	107.0 ± 2.1 <sup>c</sup>	
Tryptophan	ND	ND	ND	4.7 ± 0.5	1.8 ± 0.2 <sup>a</sup>	2.6 ± 0.4 <sup>b</sup>	1.6 ± 0.1 <sup>a</sup>	1.6 ± 0.1 <sup>a</sup>	2.5 ± 0.4 <sup>b</sup>	
Phenylalanine	31.7 ± 2.4	26.6 ± 2.0 <sup>c</sup>	25.8 ± 1.8 <sup>c</sup>	24.1 ± 3.0 <sup>bc</sup>	22.1 ± 1.7 <sup>ab</sup>	27.3 ± 2.9 <sup>c</sup>	19.4 ± 0.9 <sup>a</sup>	24.0 ± 3.7 <sup>bc</sup>	26.6 ± 3.2 <sup>c</sup>	
Histidine	21.1 ± 1.1 <sup>c</sup>	20.3 ± 0.7 <sup>c</sup>	17.5 ± 0.9 <sup>b</sup>	19.3 ± 1.5 <sup>bc</sup>	19.2 ± 1.8 <sup>bc</sup>	17.9 ± 0.9 <sup>b</sup>	14.3 ± 1.0 <sup>a</sup>	18.3 ± 0.7 <sup>bc</sup>	15.3 ± 1.3 <sup>a</sup>	
Lysine	108.2 ± 7.7 <sup>c</sup>	100.0 ± 5.4 <sup>bc</sup>	86.7 ± 4.7 <sup>a</sup>	93.5 ± 3.6 <sup>ab</sup>	86.3 ± 7.9 <sup>a</sup>	102.6 ± 4.4 <sup>bc</sup>	71.8 ± 5.3	85.2 ± 4.0 <sup>a</sup>	88.6 ± 5.2 <sup>a</sup>	
Total	996.0 ± 11.5 <sup>a</sup>	893.8 ± 12.4	762.5 ± 10.1 <sup>b</sup>	846.8 ± 1.3	698.3 ± 10.4 <sup>c</sup>	915.7 ± 10.5 <sup>a</sup>	628.6 ± 10.1 <sup>d</sup>	664.1 ± 9.8 <sup>cd</sup>	735.3 ± 8.1 <sup>b</sup>	
<i>EWP</i>										
Methionine	29.5 ± 1.5 <sup>f</sup>	28.1 ± 2.2 <sup>ef</sup>	18.0 ± 1.1 <sup>c</sup>	18.7 ± 1.8 <sup>c</sup>	13.2 ± 0.8 <sup>b</sup>	22.9 ± 2.3 <sup>de</sup>	10.0 ± 0.5 <sup>a</sup>	14.2 ± 1.8 <sup>b</sup>	11.2 ± 0.4 <sup>a</sup>	
Threonine	40.0 ± 2.9 <sup>d</sup>	37.9 ± 2.1 <sup>cd</sup>	37.9 ± 3.2 <sup>cd</sup>	34.0 ± 2.8 <sup>bc</sup>	24.1 ± 1.4 <sup>a</sup>	36.7 ± 4.2 <sup>c</sup>	25.7 ± 1.8 <sup>a</sup>	33.2 ± 2.7 <sup>b</sup>	32.0 ± 1.7 <sup>b</sup>	
Valine	59.2 ± 3.3	47.5 ± 4.6 <sup>d</sup>	33.7 ± 1.6 <sup>ab</sup>	42.2 ± 2.5 <sup>cd</sup>	31.6 ± 2.8 <sup>a</sup>	43.9 ± 1.3 <sup>d</sup>	37.4 ± 2.2 <sup>bc</sup>	41.9 ± 3.6 <sup>cd</sup>	38.8 ± 2.5 <sup>bc</sup>	
Isoleucine	46.4 ± 3.7	40.7 ± 3.5 <sup>d</sup>	26.8 ± 1.8 <sup>ab</sup>	33.4 ± 2.6 <sup>cd</sup>	26.6 ± 1.2 <sup>ab</sup>	36.3 ± 2.7 <sup>cd</sup>	32.5 ± 3.0 <sup>bc</sup>	32.3 ± 2.1 <sup>cd</sup>	35.9 ± 3.3 <sup>cd</sup>	
Leucine	75.2 ± 4.1 <sup>b</sup>	75.2 ± 5.8 <sup>b</sup>	62.8 ± 4.1 <sup>a</sup>	65.0 ± 4.4 <sup>ab</sup>	52.6 ± 2.7	73.0 ± 5.0 <sup>b</sup>	64.9 ± 4.3 <sup>ab</sup>	66.2 ± 3.6 <sup>ab</sup>	65.7 ± 4.0 <sup>ab</sup>	
Tryptophan	ND	ND	ND	9.7 ± 0.0	1.7 ± 0.1	5.7 ± 0.3 <sup>b</sup>	3.4 ± 0.2 <sup>a</sup>	5.4 ± 0.3 <sup>b</sup>	3.4 ± 0.2 <sup>a</sup>	
Phenylalanine	72.8 ± 5.5	64.1 ± 3.8 <sup>bc</sup>	56.8 ± 4.1 <sup>ab</sup>	62.8 ± 4.0 <sup>bc</sup>	51.8 ± 1.7 <sup>a</sup>	64.5 ± 2.8 <sup>c</sup>	59.0 ± 4.3 <sup>bc</sup>	64.1 ± 3.7 <sup>c</sup>	62.1 ± 5.0 <sup>bc</sup>	
Histidine	22.4 ± 1.8 <sup>b</sup>	21.5 ± 0.7 <sup>b</sup>	19.1 ± 1.1 <sup>ab</sup>	20.2 ± 1.3 <sup>b</sup>	15.2 ± 1.5 <sup>a</sup>	20.5 ± 2.4 <sup>b</sup>	18.6 ± 2.8 <sup>ab</sup>	19.5 ± 1.7 <sup>ab</sup>	19.1 ± 1.0 <sup>ab</sup>	
Lysine	57.6 ± 3.1 <sup>c</sup>	54.6 ± 2.3 <sup>bc</sup>	46.1 ± 4.5 <sup>a</sup>	49.7 ± 3.7 <sup>ab</sup>	38.2 ± 2.9	53.2 ± 4.0 <sup>bc</sup>	47.2 ± 3.3 <sup>a</sup>	49.8 ± 2.6 <sup>ab</sup>	47.9 ± 1.7 <sup>a</sup>	
Total	990.1 ± 11.9	887.7 ± 10.3	690.7 ± 7.9 <sup>c</sup>	771.0 ± 8.6 <sup>ab</sup>	576.3 ± 10.9	794.3 ± 11.2 <sup>a</sup>	689.2 ± 8.5 <sup>c</sup>	767.0 ± 11.4 <sup>b</sup>	757.1 ± 9.3 <sup>b</sup>	
<i>SPI</i>										
Methionine	18.9 ± 1.5 <sup>e</sup>	18.0 ± 1.9 <sup>e</sup>	12.1 ± 1.1 <sup>cd</sup>	15.4 ± 1.0 <sup>de</sup>	07.5 ± 0.5 <sup>ab</sup>	12.6 ± 0.7 <sup>cd</sup>	8.9 ± 1.0 <sup>bc</sup>	12.0 ± 2.2 <sup>cd</sup>	6.7 ± 0.4 <sup>a</sup>	
Threonine	39.5 ± 2.5 <sup>c</sup>	37.4 ± 3.0 <sup>bc</sup>	37.4 ± 1.8 <sup>bc</sup>	37.4 ± 1.1 <sup>bc</sup>	25.4 ± 3.6 <sup>a</sup>	33.6 ± 2.4 <sup>b</sup>	33.6 ± 2.7 <sup>b</sup>	31.6 ± 2.0 <sup>b</sup>	23.8 ± 1.5 <sup>a</sup>	
Valine	50.6 ± 3.6	40.6 ± 2.8 <sup>c</sup>	36.1 ± 2.5 <sup>bc</sup>	40.6 ± 3.3 <sup>c</sup>	32.0 ± 3.8 <sup>ab</sup>	37.5 ± 1.7 <sup>c</sup>	28.8 ± 2.0 <sup>a</sup>	35.8 ± 1.9 <sup>bc</sup>	27.0 ± 2.5 <sup>a</sup>	
Isoleucine	44.9 ± 2.9 <sup>d</sup>	39.4 ± 2.2 <sup>cd</sup>	34.7 ± 1.3 <sup>bc</sup>	39.4 ± 3.5 <sup>cd</sup>	25.9 ± 1.7 <sup>a</sup>	35.1 ± 4.4 <sup>bc</sup>	30.2 ± 2.3 <sup>ab</sup>	32.3 ± 2.8 <sup>b</sup>	25.7 ± 2.1 <sup>a</sup>	
Leucine	78.7 ± 5.4 <sup>c</sup>	78.7 ± 3.6 <sup>c</sup>	76.4 ± 2.8 <sup>c</sup>	78.7 ± 6.7 <sup>c</sup>	65.7 ± 7.3 <sup>ab</sup>	69.3 ± 4.4 <sup>b</sup>	67.9 ± 2.8 <sup>b</sup>	68.0 ± 4.9 <sup>b</sup>	55.0 ± 5.3 <sup>a</sup>	
Tryptophan	ND	ND	ND	3.6 ± 0.2	1.0 ± 0.1	2.9 ± 0.1	0.5 ± 0.1	1.7 ± 0.1	ND	
Phenylalanine	54.3 ± 3.7 <sup>b</sup>	54.3 ± 2.7 <sup>b</sup>	47.8 ± 4.3 <sup>ab</sup>	48.1 ± 3.8 <sup>ab</sup>	46.8 ± 1.8 <sup>a</sup>	47.8 ± 2.2 <sup>ab</sup>	44.0 ± 3.1 <sup>a</sup>	46.8 ± 3.7 <sup>a</sup>	38.6 ± 2.5	
Histidine	28.1 ± 1.7 <sup>d</sup>	27.0 ± 2.3 <sup>cd</sup>	23.3 ± 1.9 <sup>ab</sup>	27.0 ± 1.1 <sup>cd</sup>	24.4 ± 3.0 <sup>bc</sup>	25.7 ± 1.6 <sup>c</sup>	23.9 ± 1.8 <sup>bc</sup>	25.3 ± 1.0	19.1 ± 1.5 <sup>a</sup>	
Lysine	61.8 ± 3.7 <sup>c</sup>	58.6 ± 3.1 <sup>bc</sup>	53.4 ± 2.2 <sup>ab</sup>	58.6 ± 2.5 <sup>bc</sup>	41.0 ± 3.2	57.1 ± 3.7 <sup>bc</sup>	50.6 ± 1.7 <sup>a</sup>	53.3 ± 4.6 <sup>ab</sup>	49.5 ± 4.3 <sup>a</sup>	
Total	973.3 ± 12.7	879.3 ± 10.2 <sup>a</sup>	788.9 ± 10.0 <sup>b</sup>	873.3 ± 10.5 <sup>a</sup>	665.7 ± 9.4	786.6 ± 8.8 <sup>b</sup>	711.2 ± 9.7	748.1 ± 11.3	614.0 ± 8.7	

**Table 3** Radical-scavenging capacity, color and solubility of proteins: egg white proteins (EWP), whey protein concentrate (WPC) and soy protein isolate (SPI) interacted at pH 6.45 and 25 or 90 °C with hydroxycinnamic acids (HCAs): from green coffee extract(GCE) or as  $\beta$ -cyclodextrin inclusion complexes ( $\beta$ -CD-HCAs) or standards of caffeic acid (CA), 5-caffeoylquinic acid (5-CQA) and ferulic acid (FA);  $n = 6$ ,  $\pm$ SD, different letters in one column correspond to significant differences ( $p < 0.05$ )

Protein	HCAs preparation and temperature of interactions (°C)	Radical-scavenging capacity (%)		CIE $L^*a^*b^*$ coordinates			Solubility (%)
		DPPH'	OH'	$L^*$	$a^*$	$b^*$	
EWP	Unmodified	71.98 $\pm$ 6.45 <sup>k</sup>	81.96 $\pm$ 6.15 <sup>l,k</sup>	96.07 $\pm$ 8.17 <sup>h</sup>	-2.97 $\pm$ 0.18	17.61 $\pm$ 1.15 <sup>e,f</sup>	100.82 $\pm$ 4.13 <sup>j</sup>
	Control 25	68.78 $\pm$ 5.30 <sup>k</sup>	76.90 $\pm$ 5.65 <sup>l,j</sup>	92.49 $\pm$ 5.28 <sup>g,h</sup>	-1.90 $\pm$ 0.10	11.11 $\pm$ 0.97 <sup>c,d</sup>	98.16 $\pm$ 2.28 <sup>j</sup>
	Control 90	20.55 $\pm$ 1.90 <sup>d,e</sup>	38.83 $\pm$ 2.80 <sup>e,f</sup>	85.76 $\pm$ 7.71 <sup>e,f</sup>	-0.15 $\pm$ 0.08 <sup>b</sup>	7.95 $\pm$ 0.66 <sup>a</sup>	37.39 $\pm$ 1.55 <sup>g,h</sup>
	GCE 25	94.19 $\pm$ 7.13	97.15 $\pm$ 7.03 <sup>k</sup>	90.83 $\pm$ 6.59 <sup>f,g</sup>	-0.36 $\pm$ 0.21 <sup>b</sup>	8.17 $\pm$ 0.58 <sup>a</sup>	91.17 $\pm$ 3.16
	GCE 90	27.30 $\pm$ 1.43 <sup>g,h</sup>	40.88 $\pm$ 3.98 <sup>f</sup>	79.24 $\pm$ 5.52 <sup>d,e</sup>	1.96 $\pm$ 0.17 <sup>d</sup>	15.70 $\pm$ 0.96 <sup>e,f</sup>	24.82 $\pm$ 1.17 <sup>e,f</sup>
	$\beta$ -CD-HCAs 90	22.17 $\pm$ 1.85 <sup>e,f</sup>	37.19 $\pm$ 2.26 <sup>e,f</sup>	86.08 $\pm$ 6.50 <sup>e,f</sup>	-0.67 $\pm$ 0.04	8.56 $\pm$ 0.64 <sup>a,b</sup>	35.79 $\pm$ 1.96 <sup>g</sup>
	CA 90	29.90 $\pm$ 2.18 <sup>g,h</sup>	45.75 $\pm$ 3.18 <sup>g</sup>	82.42 $\pm$ 7.02 <sup>d,e</sup>	-0.39 $\pm$ 0.02 <sup>b</sup>	10.35 $\pm$ 0.87 <sup>c,d</sup>	21.29 $\pm$ 1.26 <sup>d,e</sup>
	5-CQA 90	24.91 $\pm$ 1.45 <sup>f,g</sup>	40.12 $\pm$ 3.42 <sup>f</sup>	77.08 $\pm$ 6.15 <sup>c,d</sup>	2.67 $\pm$ 0.19 <sup>e,f</sup>	17.56 $\pm$ 1.15 <sup>e,f</sup>	19.05 $\pm$ 1.03 <sup>d</sup>
	FA 90	28.04 $\pm$ 1.76 <sup>g,h</sup>	42.80 $\pm$ 2.70 <sup>f,g</sup>	82.35 $\pm$ 7.15 <sup>d,e</sup>	-0.86 $\pm$ 0.06 <sup>a</sup>	8.49 $\pm$ 0.83 <sup>a,b</sup>	32.21 $\pm$ 2.99 <sup>f,g</sup>
WPC	Unmodified	49.68 $\pm$ 3.43 <sup>j</sup>	59.62 $\pm$ 4.14 <sup>h</sup>	92.77 $\pm$ 6.22 <sup>g,h</sup>	-0.85 $\pm$ 0.05 <sup>a</sup>	13.35 $\pm$ 1.07 <sup>d,e</sup>	101.08 $\pm$ 3.17 <sup>j</sup>
	Control 25	37.15 $\pm$ 3.21 <sup>i</sup>	32.51 $\pm$ 2.70 <sup>d,e</sup>	95.01 $\pm$ 7.38 <sup>g,h</sup>	-0.40 $\pm$ 0.02 <sup>b</sup>	8.58 $\pm$ 0.54 <sup>a,b</sup>	97.42 $\pm$ 8.66 <sup>j</sup>
	Control 90	8.29 $\pm$ 0.57 <sup>a</sup>	19.33 $\pm$ 1.16 <sup>b,c</sup>	94.31 $\pm$ 6.55 <sup>g,h</sup>	-1.18 $\pm$ 0.03 <sup>a</sup>	8.77 $\pm$ 0.63 <sup>a,b</sup>	42.17 $\pm$ 3.19 <sup>h,i</sup>
	GCE 25	45.72 $\pm$ 3.21 <sup>j</sup>	36.12 $\pm$ 3.12 <sup>e,f</sup>	91.63 $\pm$ 4.47 <sup>f,g</sup>	0.20 $\pm$ 0.01	10.28 $\pm$ 0.94 <sup>c,d</sup>	91.55 $\pm$ 8.73
	GCE 90	13.78 $\pm$ 1.12 <sup>b,c</sup>	18.51 $\pm$ 0.87 <sup>b</sup>	82.37 $\pm$ 6.12 <sup>d,e</sup>	1.94 $\pm$ 0.12 <sup>d</sup>	23.32 $\pm$ 1.17 <sup>g</sup>	35.20 $\pm$ 5.48 <sup>g</sup>
	$\beta$ -CD-HCAs 90	18.11 $\pm$ 1.17 <sup>c,d</sup>	21.68 $\pm$ 2.08 <sup>b,c</sup>	91.77 $\pm$ 5.73 <sup>f,g</sup>	-0.39 $\pm$ 0.02 <sup>b</sup>	9.64 $\pm$ 0.83 <sup>b,c</sup>	40.39 $\pm$ 2.32 <sup>h,i</sup>
	CA 90	15.07 $\pm$ 1.46 <sup>c</sup>	21.21 $\pm$ 3.15 <sup>b,c</sup>	89.06 $\pm$ 7.89 <sup>f,g</sup>	-0.99 $\pm$ 0.06 <sup>a</sup>	18.44 $\pm$ 2.17 <sup>f,g</sup>	25.65 $\pm$ 1.55 <sup>e,f</sup>
	5-CQA 90	12.17 $\pm$ 0.98 <sup>b,c</sup>	20.03 $\pm$ 1.16 <sup>b,c</sup>	80.11 $\pm$ 4.27 <sup>d,e</sup>	2.31 $\pm$ 0.17 <sup>e</sup>	25.18 $\pm$ 2.48 <sup>g</sup>	22.43 $\pm$ 1.75 <sup>d,e</sup>
	FA 90	18.45 $\pm$ 1.34 <sup>c,d</sup>	28.23 $\pm$ 1.89 <sup>d</sup>	91.71 $\pm$ 6.65 <sup>f,g</sup>	-0.36 $\pm$ 0.02 <sup>b</sup>	12.25 $\pm$ 1.56 <sup>d,e</sup>	47.64 $\pm$ 3.6 <sup>i</sup>
SPI	Unmodified	40.25 $\pm$ 2.38 <sup>i,j</sup>	62.61 $\pm$ 4.76 <sup>h,i</sup>	84.28 $\pm$ 5.38 <sup>e,f</sup>	3.05 $\pm$ 0.28 <sup>f,g</sup>	17.70 $\pm$ 1.34 <sup>e,f</sup>	71.34 $\pm$ 6.43
	Control 25	33.15 $\pm$ 2.10 <sup>h</sup>	59.73 $\pm$ 3.74 <sup>h</sup>	74.31 $\pm$ 4.02 <sup>c</sup>	2.29 $\pm$ 0.16 <sup>e</sup>	16.42 $\pm$ 1.52 <sup>e,f</sup>	30.58 $\pm$ 2.71 <sup>f,g</sup>
	Control 90	2.87 $\pm$ 0.18	8.74 $\pm$ 0.76	66.81 $\pm$ 5.12 <sup>b,c</sup>	1.71 $\pm$ 0.11 <sup>c</sup>	15.80 $\pm$ 2.10 <sup>e,f</sup>	15.36 $\pm$ 0.53 <sup>b</sup>
	GCE 25	35.39 $\pm$ 2.08 <sup>h,i</sup>	67.08 $\pm$ 5.42 <sup>i</sup>	62.22 $\pm$ 4.77 <sup>b</sup>	2.58 $\pm$ 0.23 <sup>e,f</sup>	16.28 $\pm$ 1.78 <sup>e,f</sup>	25.05 $\pm$ 2.12 <sup>e,f</sup>
	GCE 90	7.23 $\pm$ 0.67 <sup>a</sup>	18.71 $\pm$ 2.47 <sup>b</sup>	48.28 $\pm$ 3.22 <sup>a</sup>	5.26 $\pm$ 0.43	18.90 $\pm$ 1.34 <sup>f,g</sup>	13.64 $\pm$ 1.65 <sup>a,b</sup>
	$\beta$ -CD-HCAs 90	5.36 $\pm$ 0.44	12.82 $\pm$ 1.66 <sup>a</sup>	65.19 $\pm$ 4.87 <sup>b</sup>	3.47 $\pm$ 0.26 <sup>g</sup>	14.70 $\pm$ 1.17 <sup>d,e</sup>	15.42 $\pm$ 1.98 <sup>b</sup>
	CA 90	10.79 $\pm$ 0.96 <sup>b</sup>	18.38 $\pm$ 2.01 <sup>b</sup>	64.16 $\pm$ 3.89 <sup>b</sup>	1.62 $\pm$ 0.10 <sup>c</sup>	12.32 $\pm$ 1.16 <sup>d,e</sup>	12.39 $\pm$ 1.08 <sup>a</sup>
	5-CQA 90	10.18 $\pm$ 1.26 <sup>b</sup>	13.10 $\pm$ 1.56 <sup>a</sup>	40.62 $\pm$ 5.28 <sup>a</sup>	3.05 $\pm$ 0.25 <sup>f,g</sup>	13.45 $\pm$ 0.78 <sup>d,e</sup>	11.03 $\pm$ 0.87 <sup>a</sup>
	FA 90	11.65 $\pm$ 1.96 <sup>b</sup>	22.43 $\pm$ 3.52 <sup>c,d</sup>	65.19 $\pm$ 6.14 <sup>b</sup>	1.55 $\pm$ 0.18 <sup>c</sup>	13.18 $\pm$ 1.51 <sup>d,e</sup>	13.20 $\pm$ 1.76 <sup>a,b</sup>

The results of both assays showed similar trends. Unmodified proteins revealed DPPH' and OH' radicals scavenging capacity at the level of 71.98 and 81.96 % for EWP, 49.68 and 59.62 % for WPC, and 40.25 and 62.61 % for SPI, respectively (Table 3). The highest radical-scavenging capacity was observed for EWP, and this tendency was maintained also after heating the three proteins. Heating without HCAs resulted in the reduction of DPPH' and OH' radical-scavenging capacities for EWP to 20.55 and 38.83 %, for WPC to 8.29 and 19.33 % and for SPI to 2.87 and 8.74 %, respectively. Proteins heated with HCAs formed products of interactions, which showed higher DPPH' and OH' radicals scavenge capacities than controls, in the range of 5.36–29.90 % for DPPH' and 12.82–42.80 % for OH'. It proved that even after interactions, HCAs maintained their high radical-scavenging activity and confirmed findings of many other studies on antioxidant activity of protein-HCA interaction products [37, 38].

Studies by Tsai et al. [38] and Budryn et al. [39] revealed that the temperature increase during the interactions results in a decrease in antioxidant activity, as for all proteins analyzed in this study. Such tendency is caused by degradation of polyphenols during heating, but also by degradation of sulfur-containing amino acids. Due to the fact that proteins interacted with  $\beta$ -CD-HCAs in much lesser extent than with other forms of HCAs, such products of interactions showed the radical-scavenging capacities similar to proteins heated alone, although statistical increase was observed ( $p < 0.05$ ).

#### Color of proteins after interactions with HCAs

Among the unmodified protein isolates WPC exhibited the least amount of pigments (Table 3). Heating of proteins without HCAs caused darkening, and degradation of green,



red or yellow pigments. The presence of HCAs during heating of proteins contributed to greater color changes. For all protein–GCE and protein–5-CQA interactions, a shift from green to red pigments was observed or the content of red pigments rose. Increased content of red pigments may be caused by the formation of new structures between amino acids and radical semiquinones derived from HCAs, which result in the formation of browning reaction products [40]. Among all products of protein–HCA interactions, the slightest color changes were caused by addition of HCAs in the form of inclusion complexes with  $\beta$ -CD. This is a result of limited interactions of proteins with  $\beta$ -CD–HCAs [23]. After interactions of protein isolates with HCAs in the form of GCE,  $L^*$  decreased and the order of darkening was: WPC < EWP < SPI. Darkening of protein-based films containing HCAs was earlier observed by Nuthong et al. [41] and Prodpran et al. [42]. The HCAs and SPI interactions at 90 °C decreased  $L^*$  from the initial value of 84.28 to 48.28, while SPI heated alone had  $L^* = 66.81$ . Therefore, for SPI, those differences were highly significant ( $p < 0.005$ ) and suggest the possibility of taking unattractive gray–brown color of heated food containing both SPI and free HCAs.

#### Solubility of proteins after interactions with HCAs

Except SPI (71.34 %) unmodified protein isolates were completely soluble in water (Table 3). Along with protein modifications, reduction of their solubility was observed. Decrease of solubility covered the range of 2.65–81.10 % for EWP, 3.62–77.81 % for WPC and 57.13–84.54 % for SPI. Depending on the type of protein, solubility decreased in the following order WPC < EWP < SPI and it was not correlated with AMM changes, which were the least for SPI. Thus, also other factors such as changes in isoelectric point, conformation and density of molecule could affect protein solubility [43]. Temperature rise during interactions contributed to greater solubility decrease. Depending on the form of HCAs, proteins solubility decreased in the following order:  $\beta$ -CD–HCAs < GCE < FA < CA < 5-CQA. Therefore, solubility reduction caused by interactions with HCAs was limited by formation of inclusion complexes of HCAs with  $\beta$ -CD.

#### Conclusions

The studies demonstrated that the addition of HCAs in free form to the solutions of protein isolates, such as EWP, WPC and SPI regardless heating causes an increase in molecular mass and degradation of essential amino acids as well as limits protein digestion compared with proteins processed without HCAs. Furthermore, it was reported that the HCAs presence during processing caused rise of DPPH $\cdot$  and OH $\cdot$

radical-scavenging capacity and color changes. Addition of HCAs in the form of inclusion complex with  $\beta$ -CD resulted in limitation of the protein–polyphenol interactions and as a consequence decreased changes in proteins in terms of nutritional and physicochemical properties. Taking into account (based on the literature data) high bioavailability of HCAs supplied in the form of inclusion complexes with  $\beta$ -CD, such encapsulation could be a promising solution for reducing unfavorable protein–polyphenol interactions during processing of foods enriched with HCAs.

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**Conflict of interest** None.

**Compliance with Ethics Requirements** This article does not contain any studies with human or animal subjects.

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