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Bioaccessibility of Se from Se-enriched wheat and chicken meat*

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Abstract: Selenium (Se) is an essential trace element to animals and humans as Se is incorporated in a series of organic molecules, such as 30 mammalian selenoproteins or selenoenzymes, which are vital for the basic functions of life. To increase the Se intake in Se-deficient areas, food and feed can be enriched using Se fertilizers or supplements. The aim of this study is to investigate the distribution, speciation, bioaccessibility, and bioavailability of Se in Se-enriched wheat (SW) grain and in Se-enriched chicken meat products using commercial enzymes and human gastric juices (HGJs). Results from the present work show that Se in wheat is bioaccessible and bioavailable, and that SW flour or bran can serve as a valuable dietary source of Se to humans. However, the bioaccessibility studies using commercial enzymes and HGJs for wheat flour, bran, and chicken meat digestion suggests that the use of commercial enzymes overestimate Se bioavailability. Furthermore, the use of NaCl or Tris-HCl to extract Se proteins from enriched products was not suited for bioaccessibility studies. The SW flour or bran can, however, serve as a valuable dietary source of Se to humans.

Keywords: analytical chemistry; bioaccessibility; chicken; human gastrointestinal juices; selenium; wheat.

INTRODUCTION

Selenium (Se) is an essential trace element to animals and humans, as trace levels of Se incorporated in more than 30 mammalian selenoproteins or selenoenzymes are vital for the basic functions of life [1–3]. Plants and plant products are transporters of Se from soil to humans. However, the levels of Se in agricultural products in Northern Europe are generally low, which has been attributed to poor supply of Se from soils [4,5]. In order to increase human Se intake, several strategies have been suggested. Se-enriched yeast (SY) (*Saccharomyces cervisiae*) is an attractive source of Se due to its low cost. SY can be consumed as a nutritional supplement or may be used instead of conventional yeast in baking bread [6,7]. However, the Se levels and speciation of Se in yeast may vary substantially, and the risk of toxicity exists as the absorption in humans is believed to be efficient [8]. Another strategy of improving the Se status in humans is agronomic biofortification of food products such as the Se fertilization of plant crops or administration of Se supplements to animals [11]. Inorganic Se is considered less efficient for

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uptake in human than Se organic compounds, but inorganic Se can be applied as fertilizer to plants such as wheat crops. The subsequent incorporation into selenoamino acids—in particular, selenomethionine (Se-Met) being a major selenoamino acid in grains—has proved to be efficient for uptake in humans [12]. The concentration of Se in plants can be increased by soil or foliar Se application. In this case, the application of selenate is more efficient than selenite [9,13–15]. The most efficient Se biofortification strategy for Se enrichment of cereals is by foliar application in the period of stem elongation to heading [14,16].

Selenate is transported in the xylem to growing plant parts [17,18]. The selenate uptake and retranslocation in wheat plants are competitively inhibited by sulfate (S), whereas nitrate (N) increases the Se concentration of wheat grains [14,17,19]. Thus, when the S plant availability is kept low and a split N fertilizer strategy to increase the wheat protein content is applied, the Se concentration in grains is enhanced. It is important to underline the fact that S-deficient wheat grains increase the gliadin/gluten ratio [20] and lower the bread-making quality, while increasing the Se concentration in wheat products.

Selenium is also valuable in the poultry industry as growth, sperm quality, and reproduction increase with increasing Se levels, while drip loss and lipid peroxidation during meat storage decrease [21,22]. The general agricultural practice in animal feeding is administration of selenite-enriched concentrates and mineral mixtures, even though the transfer of Se to meat, milk, and eggs is low [12]. Prooxidant properties of selenite and its interactions with dietary compounds such as ascorbic acid put pressure on feed manufacturers to explore new, more effective sources of supplemental Se. Therefore, the simplest way would be to replace selenate with Se-enriched plants in fodder as a strategy that also increases the chicken meat Se concentration [12,23].

The bioavailability of Se in food products for humans depends upon the ability of Se to dissolve in the gastrointestinal fluids and to be absorbed in the intestinal tract by crossing the intestinal cell membranes. Estimation of Se bioavailability is presently performed by using in vivo human studies, whereas the estimation of Se bioaccessibility is done by using in vitro model studies with proteolytic enzymes and/or extraction techniques. Since human experiments are time-consuming, costly, and need special ethical permission, in vitro studies using gastrointestinal juices should provide good models for mimicking human digestion to estimate the potential uptake. Therefore, the present work focuses on the distribution, speciation, bioaccessibility, and bioavailability of Se in Se-enriched wheat (SW) grains and in chicken meat products from chickens fed with SW utilizing extracting agents.

METHOD

Instrumentation

The total Se concentrations in samples and supernatants in all experiments were determined at the Norwegian University of Life Sciences, Department of Plant and Environmental Sciences (UMB-IPM) using an inductively coupled plasma-mass spectrometer (ICP-MS) (Perkin Elmer Elan 6000) after distilled ultrapure nitric acid digestion (3.5 ml) at 250 °C for 20 min using an ultraclave (UltraCLAVE 3, Milestone). The wheat N concentration was determined at UMB-IPM by using a LECO CHN analyzer (LECO Corporation, St. Joseph, MI, USA) and multiplied by 5.7 for its protein content.

Chromatographic separations of Se compounds in wheat samples were carried out at the Laboratorie de Chimie Analytique Bio-inorganique et Environnement, Pau, France, using a Model 1100 high-performance liquid chromatography (HPLC) pump (Agilent, Wilmington, DE, USA) as delivery system. The exit of the column was directly connected to the Meinhard nebulizer (Glass Expansion, Romainmotier, Switzerland) of the ICP-MS equipped with a collision cell (Agilent 7500c, Tokyo, Japan) by means of peek tubing. Injections were performed using a Rheodyne valve with 100-µl sample loop. The ICP-MS collision cell was pressurized with hydrogen, and the chromatographic mobile phases were degassed by purging with helium.

Chromatographic separation of Se compounds in chicken meat was done at UMB-IPM using a method slightly modified from that described above, as an ultrasonic nebulizer (CETAC Technologies, Inc.) coupled to the ICP-MS was used. Temperature of the cooler was 0 °C and the heater 120 °C with an argon nebulizer gas flow of 1.15 l min^{-1}.

Chromatographic conditions

The supernatant (100 µl) was injected in a Superdex 75 (300 × 10 mm) (GE Healthcare Bio-Sciences AB, Sweden) size exclusion column. Elution was isocratic using a 0.1 M ammonium acetate (pH 7.5) buffer at 0.7 ml min^{-1}. The size exclusion column was calibrated using defined standards; transferrin (81 KDa), bovine albumin (66 kDa), albumin chicken egg (44.3 kDa), superoxide dismutase Mn (40 kDa), superoxide dismutase Cu/Zn (32.5 kDa), myoglobin (16.7 kDa), MT1 (7 kDa), and vitamin B_{12} (1.6 kDa). The proteolytic supernatant was injected on a Hamilton PRPX-100 column (150 × 4.6 mm × 10 µm) anion-exchange column (Hamilton, Reno, NV, USA). Buffer A was 20 mM acetate and 10 mM triethylamine, and buffer B was 200 mM acetate and 100 mM triethylamine. The gradient used for elution was 0 % B (0–5 min isocratic), 0–100 % B (5–30 min gradient), 100 % B (30–40 min isocratic), 100–0 % B (40–41 min, gradient) 0 % B (41–45 min isocratic) at 0.7 ml min^{-1}.

Enzymatic extracting agents

Protease XIV (EC 2329095 Bacterial, *Streptomyses griseus* 5.8 U mg^{-1}), lipase (EC 23269199, *Candida rugosa* 4.01 U mg^{-1}), cellulase (EC 2327344. *Aspergillus niger* 1.4 U mg^{-1}) were obtained from Sigma-Aldrich. Pepsin (EC 232693, hog stomach 1436 U mg^{-1}), α-amylase (EC 2325656 hog pancreas 45.4 U mg^{-1}) were purchased from Fluka. Human proteolytic enzymes (HPEs) were obtained in the activated state by collecting in vivo human gastric juice (HGJ) and human duodenal juice (HDJ) [24]. In brief, a three-lumen tube enabled simultaneous installation of saline in the duodenal and aspiration of HGJ and HDJ. Saline (100 ml h^{-1}) was instilled close to the papilla of Vater and HDJ aspirated some 18 cm distally. Aspirates were collected on ice and frozen in aliquots at –20 °C. The HGJ and HDJ used in these experiments were obtained from a pooled batch of 6 individual healthy persons. Pepsin activity in the HGJ (pH 2.0) was 18.9 U ml^{-1} min^{-1}, assayed according to Sanchez-Chaing et al. [25]. Total proteolytic activity in HDJ (pH 7.0) was 16.4 U ml^{-1} min^{-1}, assayed according to Krogdahl and Holm [26]. In addition, the HDJ contained α-amylase activity of 36500 U/l and bile salt of 4.5 mM/l, analyzed at the Oestfold Trust Hospital, Norway.

Se enrichment of wheat grains and chicken feed

Cultivation of field-grown Se-enriched spring wheat (*Triticum aestivum* L. Zebra) was carried out on 0.4 ha at UMB, Aas, Norway. The experimental site was situated 70 m a.s.l. on a marine clay deposit having an annual precipitation on average 800 mm. The soil was a loam, classified as a Typic cryaquept in The Soil Taxonomy, having a pH of 5.8 (H_2O, 1:2.5) and an organic matter content in the plough layer of 5.3 %. The natural soil Se concentration was 0.29 mg kg^{-1} and plants grown in the soil had a Se concentration <0.02 mg kg^{-1} [27]. The soil was Se-enriched at seeding by using 110 kg N ha^{-1} of the Se-enriched NPK 21-3-8 fertilizer (12 mg Se as selenate kg^{-1}). At heading, plants were Se-enriched with foliar application of 34 g Se (selenate) ha^{-1}. The grain protein content was maximized by applying 30 kg N as NPK 21-4-10 ha^{-1} at heading. The grains were harvested at maturity and stored in a barn prior to milling. Ten kg of Se-enriched grains were moistened to 15 % water content and separated into flour and bran fractions at a commercial milling plant (Norgesmøllene AS). The milling fractions were stored at –20 °C prior to analysis. The SW was applied as chicken feed in a subsequent experiment.

The chicken experiment was performed at the Animal Production Experimental Centre (SHF) at UMB. Ninety newly hatched male chickens (*Ross 308*, Samvirkekylling, Norway) divided in three replicates were fed for 33 days. The chickens were placed in separate pens at random with free access to food and water. The chickens were raised in an environmentally controlled room. Chickens were fed with three different feeds; sodium selenite (SS) (0.9 mg Se kg^{-1}), SY (0.9 mg Se kg^{-1}, Sel-Plex, *Saccharomyces cervisiae* CNCM-I-3060), and the SW (1.0 mg Se kg^{-1}). All feeds used spring wheat as major energy source, and the feeds were produced at Fortek (Ås, Norway).

Extraction of Se organic compounds

Selenium organic compounds in flour and bran were extracted according to the following procedure: five samples of 0.2 g fresh weight flour and bran were extracted in 0.9 % NaCl (pH 5.5) in 2-ml Eppendorf tubes (VWR International) in a ball mill with three tungsten carbide balls (3 mm) for 3 min at 30 Hz. The extracts were centrifuged at 10 000 g × 10 min and 0.7 ml of supernatants was pooled. The extracts were added to 0.7 ml 0.9 % NaCl, and the procedure was repeated twice and supernatants pooled. The samples were stored frozen until analysis.

Proteolytic digestion

Digestion of samples was performed in a water bath at 37 °C and done in triplicate.

For the bioaccessibility experiments, 10 ml 0.9 % NaCl at pH 5.5 was added to the samples of 1 g fresh weight. In the wheat control extractions (WCEs), samples were added 25 mg lipase and 25 mg protease XIV at pH 7.5 for 480 min. In the wheat enzymatic extractions (WEDs), samples were added 2.5 mg α-amylase pH 5.5 for 10 min, 2.5 mg pepsin pH 2.0 for 120 min, and then 100 mg cellulase, 25 mg lipase, and 25 mg protease XIV pH 7.5 for 360 min. In the wheat human proteolytic extractions (WHPEs), samples were added 2.5 mg α-amylase pH 5.5 for 10 min, 100 µl HGJ pH 2 for 120 min at 37 °C, and then pH was raised to 7.5 with 1 M NaOH before the addition of 400 µl HDJ for 360 min. at 37 °C. The extracts were centrifuged at 10 000 g for 10 min and the supernatants were added 5 µl β-mercaptoethanol and stored frozen.

To obtain information on Se species in chicken meat, extraction with protease XIV and lipase was carried out (CCE). Freeze-dried chicken meat (0.1 g) was added 2 ml 7 M urea and 400 µl 0.2 M dithiolthreitol (DTT), and 10 ml with 50 mM Tris-HCl containing 30 mg protease XIV and 20 mg lipase pH 7.5, and extracted for 6 h according to a modified version of the method used by Bierla et al. [28]. The HPE extraction of chicken meat (CHPE) was performed as follows: 0.4 g of freeze-dried meat were added 10 ml of 0.9 % NaCl at pH 2.0 and 200 µl HGJ for 120 min at 37 °C. Then, pH was adjusted to pH 7.5 and 400 µl HDJ was added for 360 min; 37 °C, and centrifuged at 10 000 g (4 °C) for 10 min prior to freezing and analysis by size exclusion and total Se determination.

Bioavailability of Se in wheat

The Se-enriched whole wheat was used in the chicken feed experiment. The Se concentration in breast muscle, leg muscle, and liver were compared with that of the fodder. The bioavailability was expressed as the bioconcentration factor (BCF). BCF = Se concentration in animal product/Se concentration in fodder per day.

Method validation and quality assurance

All extractions were performed in three replicates, and chromatographic measurements were duplicated for the supernatant chosen. For the determination of the total Se concentrations in grains and the supernatants, standard reference material (SRM) wheat flour 1567A (1.1 ± 0.2 µg g^{-1}) and bovine liver

1577B (0.73 ± 0.06 μg g^{-1}) from the National Institute of Standards and Technology (NIST) were applied. The samples were digested with HNO_3 in triplicate for each of the sets of extractions and analyzed for quality assurance and control purposes. The relative standard deviation based on counting statistics was less than 2 %. Three blanks were run for each digestion procedure to improve the statistics of the measurements. Identification of Se-Met in supernatants was carried out by standard addition. In the analysis of total Se concentration in chicken, Te and In were used as internal standards.

Statistics

Comparisons of the Se concentration levels in the feed and products and the BCF (Bq/kg product/Bq/feed day) were analyzed using one-way ANOVA, Fishers LSD test at a significance level of 0.05 using Minitab statistical software [29].

RESULTS

Se concentration in wheat and chicken product samples

The Se concentration in whole wheat grain was 1.2 ± 0.2 mg Se kg^{-1}, while the concentrations in flour and bran were 1.10 ± 0.02 and 1.58 ± 0.01 mg Se kg^{-1}, respectively. Thus, the flour represented 70 % and the bran 25 % of the Se level of the whole wheat. Five percent of the whole grain was lost in the milling process. The Se concentrations in chicken breast muscle from chickens fed by Se-enriched fodder were 0.5, 1.5, and 1.9 mg Se kg^{-1} for the SS, SY, and SW, respectively. Figure 1 presents the BCF of Se to different edible chicken parts from the Se-enriched feeds. The highest BCF of Se to breast and leg was obtained with organic Se feed, including the SW feed, whereas no differences were obtained in the transfer to the liver from feed having different Se sources ($p < 0.05$).

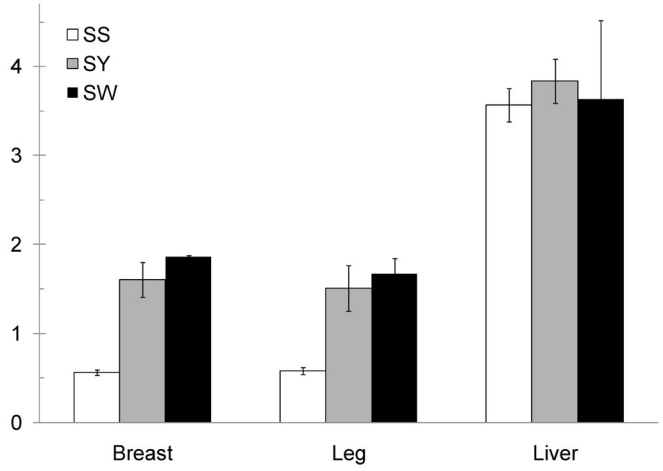

Fig. 1 Feed-to-meat ratio Se concentration in chickens fed Se-enriched feed using SS, SY, and SW.

Efficiency of extraction

The total Se concentrations in the supernatants obtained after NaCl extraction and proteolytic digestions of wheat flour, wheat bran, and the edible part of the chickens are presented in Table 1 as the percentage (%) of the total sample Se concentration. The NaCl extraction of Se proteins was higher in the bran than in flour, and the proteolytic extraction of Se species higher in the flour than in bran. The lowest

extraction yield was obtained with HPE for both wheat fractions. The highest Se yield was obtained with the WED method, whereas the WCE extraction showed the highest yield for the bran fraction. Breast muscles extracted with 5 mM Tris-HCl showed the lowest yield from the chickens fed SY. The NaCl extraction yield of the SW-fed chicken breast was similar to that obtained from the extraction using Tris-HCl.

Table 1 Se extraction yields (%) of the total Se concentration in flour, bran, and chicken breast muscle and method of speciation.

	Extraction	Yield (%)
Flour (n = 3)	0.9 % NaCl	19.1 ± 0.8
1.10 ± 0.02 mg Se g^{-1}	WCE	83.7 ± 3.3
	WHPE	75.4 ± 1.6
	WED	91.8 ± 7.2
Bran (n = 3)	0.9 % NaCl	23.3 ± 0.4
1.58 ± 0.01 µg Se g^{-1}	WCE	67.8 ± 5.0
	WHPE	51.4 ± 0.5
	WED	59.5 ± 2.6
Chicken SW (n = 3)	0.9 % NaCl	30.5 ± 2.0
1.91 ± 0.02 mg Se g^{-1}	CCE	90.2 ± 1.2
	HGJ	67 ± 4
	CHPE	76 ± 8
Chicken (SS) (n = 3)	5 mM Tris HCl	31.6 ± 2
Chicken (SY) (n = 3)	5 mM Tris HCl	13.7 ± 0.7
Chicken (SW) (n = 3)	5 mM Tris HCl	24.7 ± 1.2

The proteolytic extraction of chicken muscles was lowest in the HPE and highest using CCE. The extraction yield of Se increased from NaCl < HGJ < HDJ, demonstrating the efficiency of HDJ as extraction agent.

Speciation of Se by size exclusion

The size exclusion chromatograms (SECs) of the wheat flour and bran extracts presented in Fig. 2 showed that the flour contained Se molecules with larger molecular masses than the bran. The flour chromatogram contained seven separated peaks, whereas the bran chromatogram contained eight peaks. Both chromatograms were reproduced when spiked with Se-Met, showing the same chromatographic profile and a higher proportion of salt-soluble Se molecules eluting at the same time as Se-Met in the bran.

The SECs of the digested chicken breast meat presented in Figs. 4A–D showed that Se-containing degradation products were formed, going from high-molecular-mass (HMM) species in the NaCl extraction toward decreasing molecular masses after HGJ extraction and reaching low molecular masses (LMM) after HDJ extractions. Spiking supernatants with Se-Met confirmed the molecular mass of the Se molecules in the last peak corresponds to retention time of Se-Met in the chromatograms. The chromatogram from protease and lipase digest (D) showed one major peak eluting at the same time as expected for Se-Met.

The anion-exchange chromatograms for the determination of Se-Met in the WED extracts for the flour and bran are presented in Fig. 3. The WED flour extracts were chosen, having the highest Se concentration. The concentration of Se-Met in the flour and the bran was 46 and 40 µg l^{-1}, respectively, showing that the WED-extracted concentration of Se-Met from the flour also was higher than from the bran. The un-identified peak eluted after 2 min was also higher in the flour than in the bran.

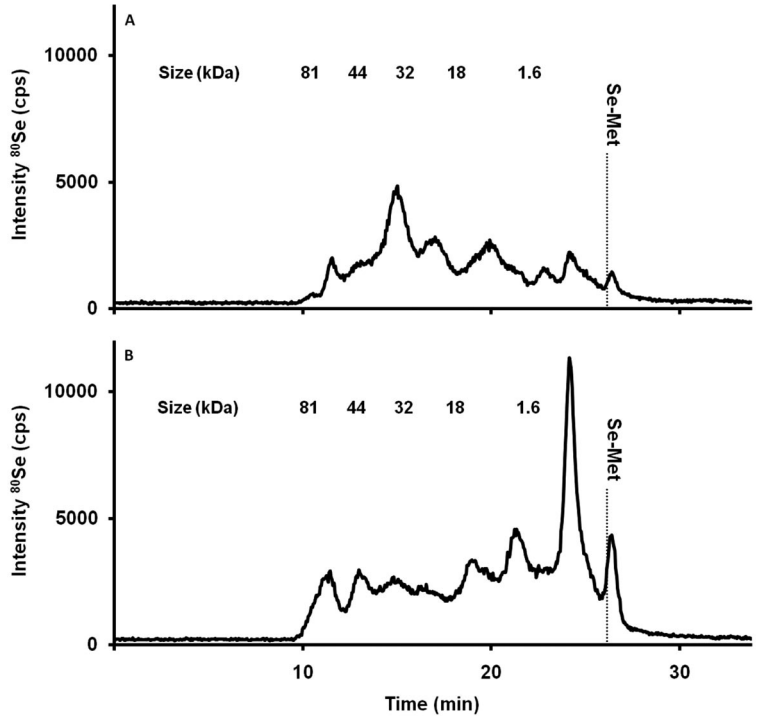

Fig. 2 SECs of 0.9 % NaCl extracted (A) wheat flour and (B) wheat bran proteins. Dotted line indicates when Se-Met eluted.

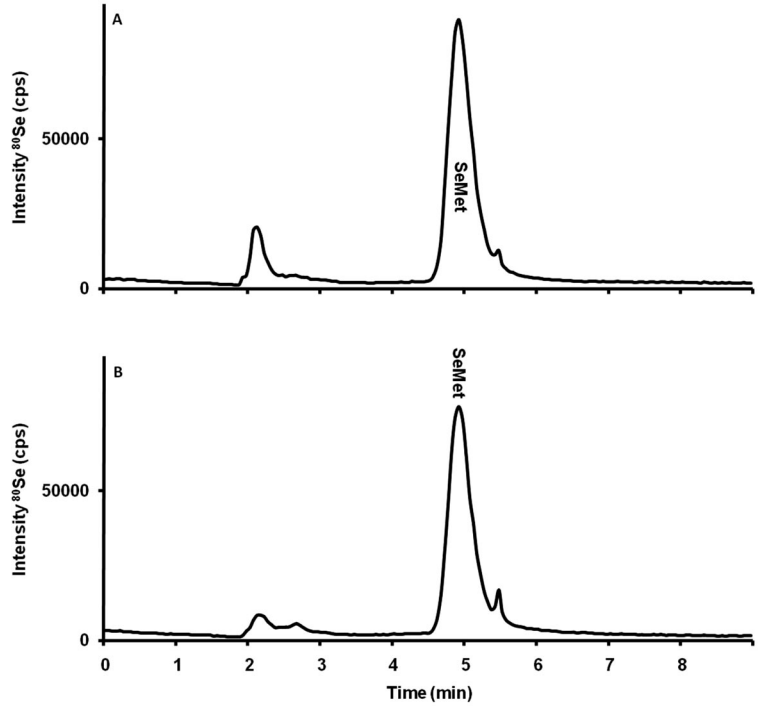

Fig. 3 Anion exchange chromatogram of Se amino acids after amylase, pepsin, protease, cellulase, and lipase extracted (A) wheat flour and (B) wheat bran.

DISCUSSION

The total Se concentrations in the NaCl extracts and the supernatants obtained after proteolytic digestion of wheat and chicken products were determined to evaluate the extraction efficiency of the different methods applied. The Se concentrations were obtained by ICP-MS using external calibration and the accuracy of the method was evaluated by analyzing biological reference materials; wheat flour 1567A and bovine liver 1577B. Since the total Se concentrations determined were within the certified values, the method used for total Se determination was considered adequate for the determination of Se in the samples.

The Se protein SEC of the wheat flour was similar to that reported by Moreno et al., having a high proportion of HMM Se organic compounds, probably proteins, in the flour [30]. The size distribution obtained for Se species in wheat represented only 20 % of the total, the remaining includes mainly water- and salt-soluble proteins. The alcohol-soluble gluten proteins, which are important for the rheological and nutritional properties of the flour, will not be present in the extracts [20]. Thus, limited information can be gained by SEC-HPLC-ICP-MS speciation technique on the distribution and bioaccessibility of Se proteins in wheat.

The main Se amino acid found in the WED extracts was Se-Met, which is in accordance with previous findings [12,31]. Selenomethionine is believed to be absorbed rapidly in the small intestine and is therefore assumed to be rather bioavailable to humans and animals [32]. In this experiment, the Se extraction yield was relatively high, but varied according to the wheat sample type and the enzymatic extraction medium applied. Wheat and meats are important dietary sources of bioavailable Se [33,34]. The flour and bran enzymatic extraction yield supported the assumption that Se in wheat is highly bioaccessible, but the yield was higher using commercial enzymes compared to the HGJs. Regardless of the enzymatic extraction medium used, a high proportion of the Se in the flour was bioaccessible and seemed to be a better Se source to humans than the bran. Since the bran has a higher Se concentration, the amount of Se available to humans was almost the same for flour and bran using the same enzymatic medium. Thus, based on the results of this Se bioaccessibility experiment, it does not matter whether humans are consuming agronomic Se-enriched flour or bran as a dietary source of Se.

The chicken breast muscle contained a factor of 3 to 4 more Se when fed organic Se than in chickens fed selenite. This was also reflected in the higher BCFs obtained, which corresponds to results reported by others [23,35]. Thus, if the goal is to maximize the Se concentration in chicken meat, organic Se should be used as Se source instead of today's practice using selenite. The results also revealed that the bioavailoability of Se in wheat is similar to Se in SY, and can be used as an alternative Se source for chickens.

Water/salt extraction yields for chicken breast were higher when NaCl was used compared to Tris-HCl, which probably was due to the high salt concentration in the NaCl extraction medium, but this needs further investigation. Only 30 % of the total Se were NaCl extracted from chickens fed SW, which is similar to findings reported by others using Tris-HCl [28,36].

The extraction yield was higher in breast meat from chickens fed selenite than for those fed organic Se, indicating that in bioaccessability studies enzymatic digestions of the whole meat fractions should be carried out, and not only, on the water/salt available fraction. Information obtained by SEC speciation is therefore limited in Se bioaccessibility studies from chicken meat. The extraction yields from chickens fed organic Se (SW) were believed to give the same results as chickens fed SY, but the SY had the lowest yield. The reason for this is not known and need further investigation.

The highest extraction yield was obtained with the CCE method, in accordance with that reported by others [28,37]. The HGJ extraction increased the Se yield to 70 %, and the extraction yield did not increase using the HDJ, which was approximately 20 % lower than acquired with the CCE extraction. Thus, the use of commercial enzymes in bioaccessibility estimations from Se-enriched meat most probably overestimates its bioavailability, although this experiment did not consider the uptake and transfer of Se molecules in the intestinal tract.

Results based on the enzymatic extraction with HPE of the chicken breast muscle (SW) and kinetics (time of reaction) as shown in Fig. 4 revealed that Se proteins are subjected to enzymatic breakdown in both HGJ and HDJ. However, the Se concentration in the supernatant did not increase between the two HPE extractions. After HGJ extraction, most Se species were degraded to molecular masses

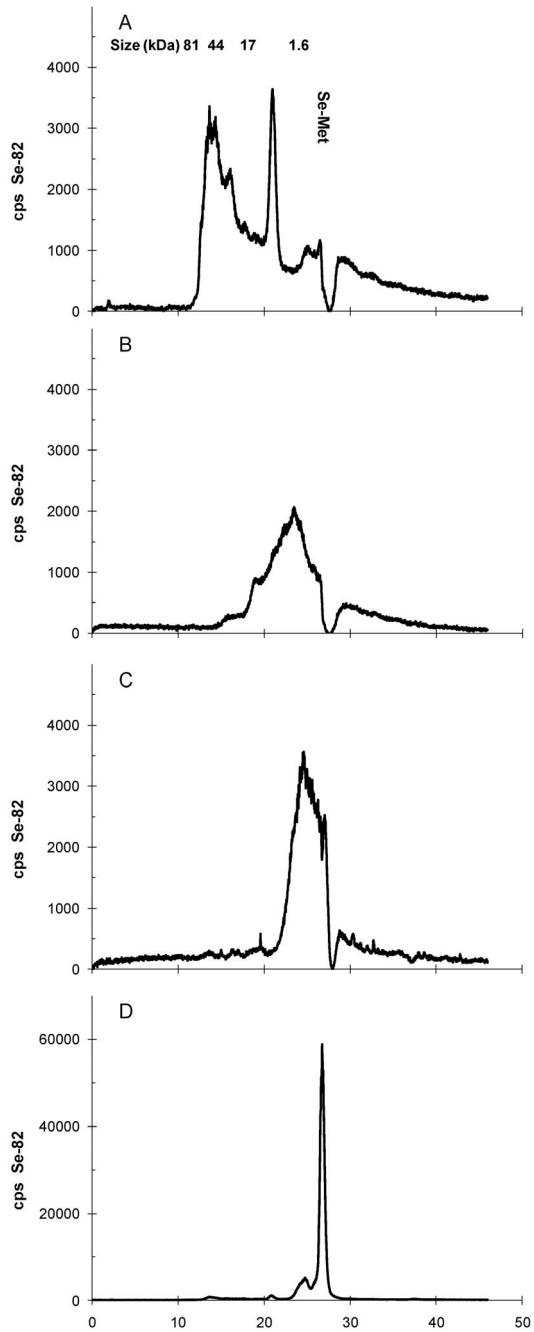

Fig. 4 SEC-ICP-MS chromatograms of different fractions of chicken breast fed SW: (A) water-soluble; (B) digested with HGJ; (C) digested with HGJ and HDJ; and (D) digested with protease XIV and lipase (CCE). ($n = 3$). Note differences in y-axis.

lower than 44 kDa, which was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS PAGE) (not shown). Because of the low resolution in the SEC and the lack of commercial Se standards, the Se species present in the HPE supernatants could not be identified and quantified. Spiking the HPE-extracted samples with Se-Met, Se-Met was eluted after 27 min. The broad peak indicates also a number of LMM Se compounds in the HPE chromatogram, in contrast to the distinct peak identified as Se-Met in the CCE. Therefore, the high bioaccessibility of Se in chicken breast muscle can be attributed a series of Se species and not only to the Se-Met as indicated by others [1,6,12,32].

A limitation in the bioaccessibility study is that humans are not consuming unprocessed wheat or chicken meat, and further studies on the effect of processing wheat and chicken products on the Se bioaccessibility and bioavailability to humans are required.

CONCLUSION

Selenium in wheat is bioaccessible and bioavailable as a feed for chickens. Thus, chicken product can be Se-enriched via Se-enriched plant feeds. Bioaccessibility studies using commercial enzymes and HGJs resulted in different extraction yields for wheat flour, bran, and meat, suggesting that commercial enzymes overestimate the Se bioavailability. As the protein distribution of Se-enriched products extracted by NaCl or Tris-HCl represented only 20–30 % of the total Se, these extraction agents are not suitable for bioaccessibility studies. The present study demonstrated, however, that SW as flour or bran can serve as a valuable dietary source of Se to humans.

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