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Metal-chelating antioxidant peptides: Biosensor screening methods as alternatives to the ferrozine assay

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Abstract

Preventing metal-catalyzed lipid oxidation in food products, which decreases nutritional value and sensory quality, is crucial in the food industry. This is typically achieved through the use of metal-chelating molecules. While the ferrozine assay is widely used to screen protein hydrolysates for metal chelating activity, it has proven difficult to use with pure peptides. This study evaluates the potential of surface plasmon resonance (SPR) and electrically switchable nanolever technology (switchSENSE[®]) as alternative screening methods. Unfortunately, solubility issues and large standard deviations precluded a direct correlation between the ferrozine assay and these biosensor techniques. Both techniques, however, were able to quantitatively distinguish between two peptides with very similar sequences despite the absence of a correlation between dissociation constants determined by SPR and switchSENSE[®]. This study highlights the potential of SPR and switchSENSE[®] for screening the metal chelating activity of pure peptides, advancing the understanding of peptide-metal ion interactions.

KEYWORDS

antioxidants, biosensor, chelation, lipid oxidation, peptides, surface plasmon resonance, switchSENSE[®]

INTRODUCTION

Lipid oxidation of food products is a major problem in the food industry. It results in a decrease in nutritional value and sensory quality (McClements & Decker, 2017) and has been shown to cause adverse health effects such as colonic inflammation in mice (Zhang et al., 2019). The changes in sensory properties may lead to a drastic decrease in the shelf life of food products and ultimately to increased food waste (Decker & Bayram, 2021).

Lipid oxidation is, among other factors, catalyzed by the presence of transition metal ions, such as Fe³⁺/Fe²⁺ (Frankel, 2005). This phenomenon happens in two ways: (i) metal ions initiate oxidation by reacting directly with unsaturated fatty acids to generate highly reactive lipid radicals or (ii) metal ions catalyze the decomposition of

lipid hydroperoxides into alkoxy or peroxy radicals. The lipid radicals and peroxy radicals are part of the propagation step in lipid oxidation. The alkoxy radicals may undergo β-scission to produce the secondary volatile oxidation products responsible for off-flavors and unwanted aromas.

Iron ions (Fe²⁺/Fe³⁺) are present in trace amounts in many foodstuffs. Egg-based oil-in-water emulsions, such as mayonnaise, are particularly susceptible to metal-catalyzed oxidation due to the iron content of egg yolk (Jacobsen, 1999). Thus, metal-chelating antioxidants are added to food products to sequester the metal ions, preventing them from acting as catalysts of lipid oxidation. To date, one of the most efficient metal chelators used in the food industry is ethylenediamine-tetraacetic acid (EDTA). However, EDTA is synthetic,

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and some health concerns are associated with its use. Due to increasing consumer demand for natural, plant-based and “label-friendly” ingredients (Asioli et al., 2017), it is of great interest to find natural alternatives to EDTA, which is close to – or as efficient – in preventing metal-catalyzed oxidation in products such as mayonnaise.

Metal-chelating peptides have been identified in, and extracted from, sustainable sources such as soybean, pea, potato, and seaweed (El Hajj et al., 2023; Irankunda et al., 2024; Lv et al., 2009; Yesiltas et al., 2022). Traditionally, potential metal chelators have been screened using time-consuming storage experiments or assays such as the ferrozine assay. The ferrozine assay is widely used for protein hydrolysates (Farvin et al., 2010; Hamit et al., 2022; Vavrusova et al., 2015). However, it has proven difficult to use it when screening pure synthetic peptides (Yesiltas et al., 2022) due to their poor solubility or gelling phenomenon. Recently, techniques such as surface plasmon resonance (SPR) and electrically switchable nanolever technology (switchSENSE[®]) have been adapted to screen hydrolysates for the presence of metal-chelating peptides (MCPs) (Canabady-Rochelle et al., 2018; El Hajj et al., 2023) as well as investigating the affinity of synthetic peptides for immobilized Ni²⁺. In this study, we utilize these two technologies for screening MCPs. The SPR technology is an optical technique extensively utilized for label-free determination of dissociation constants (K_D) in ligand-analyte interactions. The ligand (metal ion here) is immobilized and the analyte (the peptide in our case), initially circulating in solution, tends to form a complex with the immobilized ligand according to its affinity for the ligand. This peptide-metal ion complex formation induces a change in refractive index near the gold plasmon, resulting in a measurable shift of the resonance angle of polarized light reflected through a prism. The shift in resonance angle is quantified as resonance units (RU) as a function of time, generating a so-called sensorgram.

By examining various concentrations of the analyte and determining the resonance value at equilibrium, a sorption isotherm can be constructed, representing the equilibrium resonance as a function of peptide concentration. The resulting sorption isotherm typically exhibits a hyperbolic curve, from which the maximum resonance value at saturation (R_{max}) and the dissociation constant (K_D , M) corresponding to the concentration determined at half of R_{max} can be determined. Notably, the U-shaped configuration of the SPR chip, consisting of two channels, allows for simultaneous evaluation of specific peptide-metal ion interactions while subtracting non-specific interactions (signal obtained from the channel lacking immobilized Ni²⁺).

The switchSENSE[®] is a technology based on electro-switchable DNA nanolevers and is used to study biomolecule interactions in real time between

proteins immobilized on the nanolevers and analytes such as proteins, nucleic acids, or small molecules. Electrically actuated DNA nanolevers are end-tethered to gold microelectrodes, which are arranged in sextuplets within four individual flow channels. The two first electrodes (E1 and E2) are the reference spots while the four other electrodes (E3 to E6) are the sample spots. A Cy5 fluorescent probe is bound to one of the DNA strands, while the analyte binds to the other DNA strand. Narmuratova et al. (2022) employed switchSENSE[®] to investigate the relationship between antioxidant properties and chelation of divalent metal ions (Fe²⁺, Zn²⁺, Cu²⁺, and Ca²⁺). They immobilized the analyte of interest, equine lactoferrin, to the DNA strand while circulating the metal ions.

In the present work, the SPR and switchSENSE[®] technologies were employed with immobilized nickel ions as ligands and free peptides as analytes. In both techniques, nitrilotriacetic acid (NTA) was used as the capture molecule to immobilize Ni²⁺. Ni²⁺ was used as neither of the techniques have been developed using Fe³⁺/Fe²⁺. To develop both techniques on Fe²⁺, a long-time optimization should be carried out in terms of metal ion solution, buffer, and other physico-chemical parameters such as pH. Instead, we considered the methodologies already developed on Ni²⁺ (Canabady-Rochelle et al., 2018; El Hajj et al., 2021). Additionally, Ni²⁺ and Fe²⁺ carry the same charge and have the same coordination number. They have similar electro-negativities and are both considered intermediate Lewis acids in Pearson's Hard and Soft Acids and Bases (HSAB) theory. Considering the potential of MCPs as alternatives to EDTA to protect against lipid oxidation, this study aimed to (i) investigate the potential of SPR and switchSENSE[®] as screening techniques for metal-chelating activity of synthetic peptides and (ii) compare the obtained results with the classical ferrozine assay.

MATERIALS AND METHODS

Materials

The peptides selected for this study were known to be embedded in potato protein. They were selected using two different models: an empirical-based bioinformatics approach (AnOxPePred) (Olsen et al., 2020) and a theoretical model for metal chelation based on HSAB theory, recently developed by the University of Lorraine. For the HSAB modeling, the 10 most abundant proteins in potatoes were selected (UniProtKB: PATB2, PAT07, Q3S488, PT3K1, PT2K1, PT1K2, Q2XPY0, M1AMY4, SPI2, Q8H9D6). These protein sequences were then subjected to theoretical enzymatic hydrolysis by trypsin, alcalase[®], protamex[®], alcalase[®] sequentially followed by flavourzyme[®] or

TABLE 1 Physicochemical characteristics and theoretical chelation properties of synthetic peptides.

Amino acid sequence	Length	Theoretical hydrolysis enzyme	Net charge		Average hydrophilicity ^a	AnOxPePred chelation score	HSAB Ni ²⁺	HSAB Fe ²⁺	HSAB Fe ³⁺
			pI ^a	at pH 7 ^a					
MDDAS	5	Trypsin	2.91	-2	0.9	0.23	-	-	-
DNHETYE	7	Alcalase	3.77	-2.91	0.86	0.27	Excellent	Moderate	Excellent
ASH	3	Trypsin	7.9	0.09	-0.23	0.28	Excellent	Moderate	-
DLHSQNNY	8	Alcalase	4.95	-0.91	-0.09	0.31	Excellent	Moderate	Moderate
DHGPKIFEPS	10	Alcalase	5.21	-0.91	0.45	0.22	Excellent	Moderate	Moderate
THTAQETAK	9	Trypsin	7.88	0.09	0.39	0.23	Excellent	Moderate	Moderate
THTAEETAK	9	Trypsin	5.31	-0.91	0.70	0.22	Moderate	Moderate	Good
HHHHHH	6	-	8.19	0.54	-0.5	0.39	-	-	-

^aCalculated using peptide calculator (BACHEM, Bubendorf, Switzerland).

protamex[®] sequentially followed by flavourzyme[®]. The specificity of the used pure enzymes is classically known from literature. Note that Alcalase[®] and Protamex[®] are two serine endopeptidases obtained from *Bacillus*. Both comprise the so-called “Subtilisin” enzyme, which has a broad hydrolyzing specificity. Being an exopeptidase, Flavourzyme[®] is widely used as a second protease in sequential hydrolysis studies with an endopeptidase being the first hydrolyzing enzyme (El Hajj et al., 2023). The theoretical enzymatic hydrolysis was based on a chemical reaction assisted by computer according to the same enzymatic rules of cleavage reported for the previous enzymes in the literature. The resulting predicted peptides were then ranked according to the HSAB theory as a function of their potential (moderate, good, or excellent) to chelate Ni²⁺, Fe²⁺, or Fe³⁺, depending on their amino acid composition and sequence. Six different peptides (i.e., ASH, THTAEETAK, THTAQETAK, DHGPKIFEPS, DLHSQNNY, DNHETYE) were selected since they were predicted to be excellent Ni²⁺ chelators in addition to moderate or good Fe²⁺ or Fe³⁺ chelators. The peptide MDDAS has previously been predicted using AnOxPePred to be a good metal chelator and was included as well (Yesiltas et al., 2022). Finally, the peptide HHHHHH was included as a positive control due to its high affinity for Ni²⁺ and its common use as tag for protein purification using Ni²⁺-Immobilized Metal Affinity Chromatography (IMAC) (Bornhorst & Falke, 2000). Once selected, peptides were synthesized by GeneCust (Boynes, France) to a certified purity of >95%. The physicochemical properties of the peptides and their bioinformatics scores are shown in Table 1. The chemicals and solvents used were of analytical grade.

Fe²⁺ chelation using the ferrozine assay

The ability of the synthetic peptides to chelate Fe²⁺ in solution using the ferrozine assay was determined according to Yesiltas et al. (2022), with some modifications. Several of the selected (MDDAS, DNHETYE, DHGPKIFEPS,

and DLHSQNNY) peptides were not soluble in water at 5000 μM. This was surprising since MDDAS, DNHETYE, and DHGPKIFEPS had average hydrophilicity values above 0.45 indicating that they are dominated by hydrophilic amino acids (Table 1). HHHHHH, which had the lowest average hydrophilicity of -0.5, dissolved in water without issues. This underscores that peptide solubility is not solely dependent on hydrophilicity. For that reason, the peptides were dissolved in pure dimethylsulfoxide (DMSO) at different concentrations (312.5 to 5000 μM). The peptide HHHHHH was dissolved in both water and DMSO to investigate the solvent effect. One hundred microliter of peptide solution were mixed with 110 μL water in 96-well microtiter plate. Twenty microliter of 0.5 mM aqueous FeCl₂ was added, and the solution in each of the wells was mixed with a pipette. The microtiter plate was shaken for 3 min at 500 rpm on ThermoMixer C (Eppendorf, Hamburg, Germany) and left to react for 3 or 30 min in the dark at room temperature. After reaction with iron, 20 μL of 2.5 mM aqueous ferrozine were added and the solution in each well was mixed with a pipette. The plate was shaken again for 3 min at 500 rpm and left to stand in the dark for 10 min. Then, the absorbance was measured at a wavelength of 562 nm using a BioTek Eon Microplate Spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The iron chelation was calculated as shown in Equation (1).

$$\text{Iron chelation (\%)} = \left(1 - \frac{A_s - A_0}{A_B}\right) \times 100 \quad (1)$$

where: A₀ is the absorbance of the blank (iron and ferrozine only, the volume of sample was replaced by water/DMSO), A_s is the absorbance of the sample (with peptides), and A_B is the absorbance of the blind (without reagents, volume of reagents replaced by water/DMSO).

In all experiments, ethylenediaminetetraacetic acid (EDTA) was investigated as a positive control. EDTA was added at 60 μM, which corresponds to roughly 60% chelation. All measurements were done in triplicate.

Surface plasmon resonance

The affinity between the peptides and immobilized Ni^{2+} was analyzed by surface plasmon resonance (SPR). The analysis was carried out on a Biacore X100 instrument (GE Healthcare, Uppsala, Sweden) equipped with a NTA (nitriloacetic acid) sensor chip at 25°C according to Canabady-Rochelle et al. (2018) and adapted from Knecht et al. (2009). Binding experiments were performed at a flow rate of 20 $\mu\text{L min}^{-1}$. Ni^{2+} was loaded onto the NTA chip using a NiCl_2 solution (500 μM , Biacore kit, Uppsala, Sweden) for 1 min followed by 1 min of stabilization period. A NTA flow cell without Ni^{2+} was used as the reference to determine non-specific interactions. The running buffer (PBS 1X, pH 7.4, 0.005% Tween[®] 20) was prepared from a PBS 10X stock buffer (67 mM $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 12.5 mM KH_2PO_4 , 150 mM NaCl with a pH of 6.65). The PBS 10X was diluted 1:10 and the pH was adjusted to 7.4 using NaOH or HCl. Then, 50 μL of Tween[®] 20 were added to 1 L of PBS 1X. Peptides were dissolved in freshly prepared PBS 1X at seven different concentrations before each experiment. A concentration range was prepared using serial dilution of a stock solution. The concentration of the stock solution was typically 10,000 μM and the initial concentration range was 250 to 10,000 μM . The concentration range was adapted between experiments based on the results. The goal was to obtain a hyperbolic profile to determine R_{max} . Each peptide concentration was injected into both flow paths for 270 s, corresponding to association, followed by 270 s of dissociation. Between each investigated peptide concentration, the chip surface was regenerated with a 500 mM imidazole solution followed by a first regeneration solution (10 mL PBS 1X, 87 μL of 350 mM EDTA). Regeneration of the chip was carried out by 1 min injection of 500 mM imidazole solution followed by a washing step with 350 mM EDTA solution. The surface was then washed with a SDS solution (0.5% v/v) for 1 min at 40 $\mu\text{L min}^{-1}$ followed by an extra-wash with running buffer. Each regeneration and washing step were repeated twice. One buffer blank before and after each sample series were also investigated for double referencing during data processing.

The obtained sensorgrams were processed with the BIAevaluate[®] software from Cytiva (Uppsala, Sweden) to get the sorption isotherms (Resonance Unit, RU; corrected by the offset value), plotted as a function of the concentration of peptide. For each peptide experiment, the response from the blank run was subtracted and the equilibrium dissociation constant (K_D , M) was determined by fitting the experimental data with the 1:1 binding model (Canabady-Rochelle et al., 2018).

Electrically switchable nanolever technology (switchSENSE[®])

All seven peptides were dissolved in T40 buffer (10 mM Tris-HCl buffer, pH 7.4, containing 40 mM NaCl, and

0.05% (v/v) Tween[®] 20) at 1 mM. The switchSENSE[®] experiments were carried out on a DRX Biosensor Analyzer (SwitchSENSE[®] Dynamic Biosensors GmbH, Planegg, Germany) according to the method developed by El Hajj et al. (2021) with some modifications. An electro-switchable DNA chip MPC-48-2-R1-S containing tris-nitrilotriacetic acid and the fluorescent probe Cy5 were used and Ni^{2+} loading was done according to manufacturer instructions. The chip is composed of six electrodes connected to two reference spots (E1, E2) and four sample spots (E3-E6) carrying the Ni^{2+} functionalized acid (Tri-NTA)-tagged DNA nanolevers.

Three concentrations of each peptide (0.1–10 μM) were injected in the microfluidic device at a flow rate of 30 $\mu\text{L min}^{-1}$ for 4 min (association phase). Then, T40 buffer was injected at 50 $\mu\text{L min}^{-1}$ for 4 min to release the analytes (dissociation phase). After investigating a peptide concentration, the immobilized Ni^{2+} ions were removed using 100 mM EDTA followed by regeneration with Ni^{2+} before studying another peptide concentration. For the kinetic experiments (static measurements), a blank control was measured using peptide-free buffer and subtracted to correct the sample signal. Between the association and the dissociation phases, the flow was stopped, and dynamic measurements were performed for all samples to determine the interaction between the metal ion and the peptide, and to analyze the size of the complex formed, as described by El Hajj et al. (2021). The kinetic parameters k_{on} , k_{off} , and the resulting K_D ($k_{\text{off}}/k_{\text{on}}$) were obtained by nonlinear fitting of the curves with single-exponential functions using the switch-ANALYSIS[®] software from Dynamic Biosensors (Munich, Germany). The error presented with the results corresponds to the global fit error of all measurements. Each experiment was performed twice.

Statistical analysis

Data were expressed as mean \pm standard deviation. Data analysis was done using the Statgraphics[®] Centurion 18 software (Statistical Graphics Corp., Rockville, MD, USA). First, multiple sample comparison analysis was performed to identify significant differences between the samples. Second, mean values were compared using Tukey's test. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Ferrozine assay

Ferrozine assay is often used to investigate iron chelation and determine the concentration of a sample, which results in 50% chelation of iron (EC_{50}) (Farvin & Jacobsen, 2013; Hermund et al., 2018). In this study,

however, it was not possible to determine the EC_{50} value since none of the samples reached 50% chelation. For all the studied peptides, except for HHHHHH, there was little or no increase in % iron chelation when increasing the concentration from 312 μM to 5000 μM and chelation did not exceed 10%. Additionally, the relative standard deviations associated with the results often exceed 100% making it impossible to trust them. The positive control, EDTA, displayed an average chelation efficiency of 80% in DMSO, surpassing the expected 60%, suggesting that solvent properties significantly influence chelation measurements and that peptides might exhibit lower chelation in water. This could be because DMSO can act as a ligand and bind some of the available iron ions in a complex (Sakiyama, 2022). The peptide HHHHHH showed more promise, reaching a chelation of $24 \pm 7.5\%$ at 5000 μM , when using water as a solvent in a preliminary experiment. Based on this, we investigated if there was a difference when increasing the incubation time from 3 to 30 min and if there was a difference between water and DMSO solvent for HHHHHH in an additional experiment. When dissolving HHHHHH in water, the chelation at 5000 μM was $20.9 \pm 3.1\%$ and $16.0 \pm 2.4\%$ for 3 min and 30 min, respectively. When dissolving HHHHHH in DMSO, the chelation at 5000 μM was $6.3 \pm 1.2\%$ and $7.2 \pm 0.7\%$ for 3 min and 30 min, respectively. This was unexpected, since the opposite trend was observed for EDTA where the chelation was the highest in DMSO. There was no significant effect of the incubation time (3 or 30 min), irrespective of the solvent used (water or DMSO). However, when DMSO was used as a solvent, chelation was significantly lower for HHHHHH compared to using water as the solvent. This suggests that solvent has a larger impact on the chelation than the incubation time. When repeating the 3 min DMSO sample, a significant decrease in chelation was observed. This highlights the inconsistency of the assay when analyzing synthetic peptides. Additionally, none of the treatments resulted in chelation exceeding 24% in the range of investigated concentrations. Given that HHHHHH has a high affinity for Ni^{2+} (K_D of 1 μM) (Knecht et al., 2009), it is surprising that it is not possible to reach higher chelation than 24%. Yesiltas et al. (2022) studied synthetic peptides predicted to be metal chelators. They dissolved the peptides in pure DMSO, which improved the solubility, but some of their peptide solutions were still cloudy indicating solubility issues. They suggested that the isoelectric point (pI) of the peptides could play a role in the solubility. When the pH of a solution is equal to the pI, the peptide has a net charge equal to 0 and is the least soluble. Both ASH and THTAQETAK had a net charge of 0.09 at pH 7, however, both peptides were completely soluble in water. The inconsistent results of the ferrozine assay in both the present study and the one by Yesiltas et al. (2022) emphasize the need for other methods to investigate the metal-chelating activity of synthetic peptides.

Surface plasmon resonance

Four of the seven investigated peptides (MDDAS, DNHETYE, DHGPKIFEPS and DLHSQNNY) had solubility issues in the PBS buffer at pH 7.4. This was alleviated by adding NaOH to the stock solution of three of these peptides: DHGPKIFEPS, DNHETYE, and DLHSQNNY. Five percent (v/v) of 0.1M NaOH was added to DHGPKIFEPS and 5% 1M NaOH was added to DNHETYE and DLHSQNNY. Yet, it was not possible to fully solubilize MDDAS. For this latter peptide, the insoluble parts were removed using the 0.22 μm syringe filter and the sample was analyzed. This filtration would most likely reduce the number of peptides available for binding to Ni^{2+} . Despite this, MDDAS was included to underscore some of the difficulties associated when analyzing synthetic peptides in SPR. Furthermore, the addition of NaOH could affect the bulk refractive index during the analysis. However, since it was added to the peptide stock solution, which were then diluted, the effect should be the same for all seven samples. Additionally, the use of a reference channel further compensates for any effect caused by adding NaOH. Finally, the addition of NaOH could also lead to slightly different pH for all the diluted samples. Yet, since the experiments were run at pH 7.4, the difference was most likely negligible. Both factors are important to consider when interpreting the results.

Figure 1 shows the sorption isotherms obtained for the seven peptides. Apart from MDDAS and THTAEETAK, the isotherms generally present a hyperbolic profile indicating saturation, or tendency to saturation, at higher concentrations (plateau value). For ASH, DLHSQNNY, DHGPKIFEPS, DNHETYE, and THTAQETAK the responses were concentration-dependent with a tendency of saturation at higher concentrations indicating peptide affinity for Ni^{2+} . Inversely, for THTAEETAK and MDDAS peptides, no affinity for Ni^{2+} was observed. The SPR binding parameters obtained after fitting the sensorgram are shown in Table 2. Due to poor fitting of the experimental data, it was not possible to reach K_D standard errors (SE) below 100% for DNHETYE and THTAQETAK. It might be possible to obtain lower standard deviations by further optimizing the concentration ranges. Yet, this would require higher concentrations in order to reach saturation, which might not be possible due to the solubility issues of some of the peptides.

The obtained K_D values for ASH, DHGPKIFEPS and DLHSQNNY were 62, 77, and 3558 μM , respectively (Table 2). In comparison, the Ni^{2+} /EDTA complex in aqueous solution has a K_D of 4×10^{-13} μM (Martell et al., 2001). It is important to note that, according to the equipment specification, if the relative SE associated with the K_D is higher than 10%, we cannot trust the precise K_D value, but rather the range that the value is in.

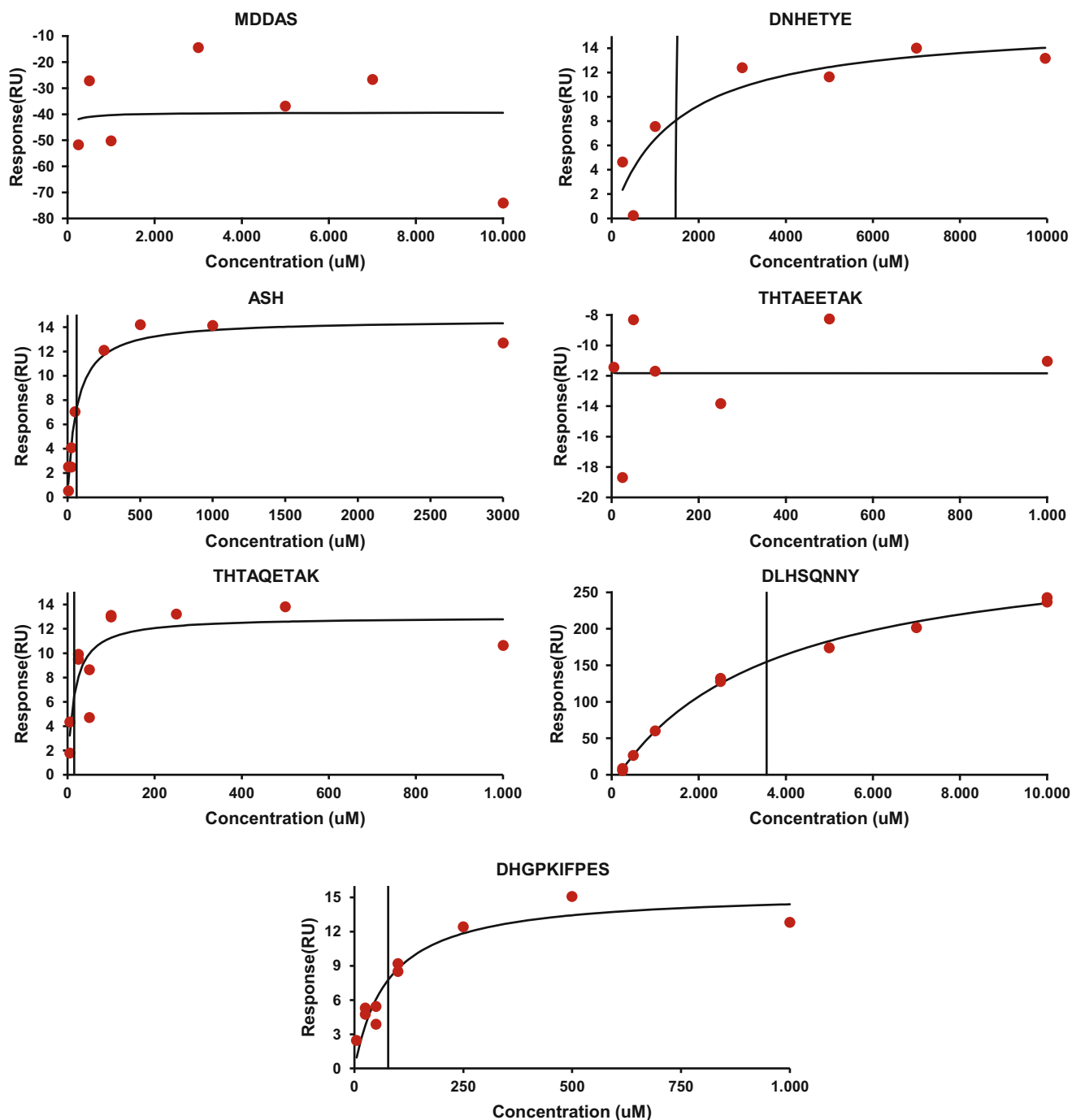


FIGURE 1 Sorption isotherms for the seven analyzed peptides. The vertical line represents the K_D . Data corrected by the offset.

The K_D values for ASH and DHGPKIFEPS were not significantly different from each other, but significantly lower than the K_D value of DLHSQNNY, meaning that ASH and DHGPKIFEPS had a better affinity for Ni^{2+} than the DLHSQNNY peptide had. Interestingly, DLHSQNNY also had the lowest relative standard deviation compared to the other samples due to a better fit of the experimental data. The obtained K_D values are within the same range as results obtained with synthetic peptides from previous studies using

this methodology (Knecht et al., 2009; Muhr et al., 2020). Knecht et al. (2009) studied the interaction between different oligohistidine (2–10 His residues), His_2Ala_4 and His_xAla_x peptides on immobilized Ni^{2+} at pH 7.4 using SPR. They found that an oligohistidine peptide with six histidine residues had the lowest K_D (0.014 μM). The HH dipeptide had a K_D of 62.7 μM , which is similar to the values obtained in this study for ASH and DHGPKIFEPS. The various HisAla peptides had K_D values ranging from 6.10 to 440 μM

TABLE 2 Binding parameters from surface plasmon resonance and switchSENSE analysis. For SPR, the equilibrium dissociation constant (K_D) and the associated standard error of the fit (SE) are shown. For switchSENSE, the kinetic rate constants (k_{on} , k_{off}) and K_D are shown \pm the error of the fit. [ND] indicates not determined in the investigated concentration ranges. [*] indicates poor fit of the experimental data.

Sample	SPR		switchSENSE		
	K_D (μM)	SE (μM)	k_{on} ($\text{M}^{-1} \text{s}^{-1}$)	k_{off} (s^{-1})	K_D (μM)
MDDAS	ND	ND	$14,300 \pm 3800$	0.11 ± 0.02	7.54 ± 2.26
DNHETYE	1468	2000*	$11,200 \pm 1400$	0.04 ± 0.03	3.72 ± 2.58
ASH	62	26	$33,000 \pm 5300$	0.06 ± 0.01	1.92 ± 0.52
THTAEETAK	ND	ND	$16,300 \pm 4400$	0.14 ± 0.05	8.49 ± 3.84
THTAQETAK	15	17	$23,000 \pm 3500$	0.02 ± 0.01	1.02 ± 0.52
DLHSQNNY	3558	470	6000 ± 630	0.005 ± 0.0013	0.81 ± 0.24
DHGPKIFEPS	77	33	5730 ± 770	0.01 ± 0.01	2.28 ± 1.33

for HAAHAH and AHAAA, respectively. Interestingly, they obtained a K_D of $175 \mu\text{M}$ for AHH compared to 62 for ASH in this study. Serine has previously been reported to be involved in chelation of Fe^{3+} through its hydroxyl group (Lv et al., 2009) and this could have had a positive effect on nickel binding as well. It is important to note that the study carried out by Knecht et al. (2009) used a HEPES buffer compared to the PBS buffer used in the present study. This should, however, not affect the results as HEPES has negligible binding of metal ions (Xiao et al., 2020). Muhr et al. (2020) studied the relationship between K_D determined by SPR and the retention time in Immobilized Metal Affinity Chromatography. They analyzed 10 tripeptides, including HHH, using the same SPR method used by Canabady-Rochelle et al. (2018). The K_D values obtained ranged from 69 to $6210 \mu\text{M}$ for HHH and RTH, respectively. They obtained a K_D of $5590 \mu\text{M}$ for the DSH peptide. This latter peptide is similar to ASH ($K_D = 62 \mu\text{M}$) from our present study. Interestingly, adding a deprotonated carboxyl group in form of aspartic acid (D) in place of the methyl group from alanine (A) increased the K_D and thus decreased the affinity of DSH for Ni^{2+} compared to ASH. This could potentially be due to a steric effect of the bulkier aspartic acid residue compared to alanine. Additionally, when looking at THTAQETAK and THTAEETAK, the presence of glutamine (Q) instead of glutamic acid (E) seems to increase the affinity for Ni^{2+} . This result is unexpected since the carboxyl group of glutamic acid is fully deprotonated at pH 7.4 and thus should favor the binding onto the Ni^{2+} . Yet another key factor in peptide-metal ion interaction is the position of each amino acid residue within the peptide sequence and thus, the possibility of steric hindrance (Knecht et al., 2009). The two glutamic acid residues in THTAEETAK might be too close, which could be unfavorable for Ni^{2+} binding. However, in the case of THTAEETAK and THTAQETAK, where $-\text{NH}_2$ and $-\text{OH}$ groups constitute the only difference in the

peptide sequence, suggests that the difference in affinity for Ni^{2+} does not arise from steric hindrance, but rather from the functionality of the amino acid. The SPR binding parameters obtained in this study are associated with large standard errors, and as such, it is difficult to conclude if one peptide is better than the other one. However, from the obtained isotherms, the SPR technique has potential to be used as a screening technique for selecting peptides for further study regarding their metal-chelating properties.

Electrically switchable nanolever technology (switchSENSE[®])

Static measurements

The static measurements or “proximity sensing” mode in switchSENSE[®] uses the quenching of fluorescence of Cy5 fluorophore in close proximity of Ni^{2+} . When the Ni^{2+} is immobilized onto the DNA nanolever, this metal ion quenches the fluorescence of the neighboring fluorophore (Cy5). Yet, when a peptide analyte binds to the nickel ion, the latter is hidden and an overall increase in fluorescence is observed. During the dissociation step of peptide from Ni^{2+} , the Cy5-fluorophore becomes closer to Ni^{2+} , thus the fluorescence decreases again. The association and dissociation rate constants (k_{on} , k_{off}) are determined by global, non-linear fitting of the association and dissociation data and the dissociation constant (K_D) is then calculated as k_{off}/k_{on} .

The results from the static measurements are shown in Table 2. Association rates (k_{on}), dissociation rates (k_{off}) and equilibrium dissociation constants (K_D) were determined for all the seven peptides. The K_D values ranged from 0.81 to $8.49 \mu\text{M}$, with the lowest and the highest K_D values obtained for DLHSQNNY and THTAEETAK, respectively. As such, all investigated peptides were able to bind to the immobilized Ni^{2+} . In general, it was difficult to

accurately fit the association and dissociation experimental curves (Figure 2) and therefore, to precisely determine the kinetic rate constants (k_{on} , k_{off}) and thus the resulting dissociation constants (K_D). This could be attributed to the fact that switchSENSE[®] was primarily designed to study interactions of larger molecules such as proteins and antibodies as opposed to the smaller peptides analyzed in the present study. This suggests that switchSENSE[®] could have limitations in terms of precisely characterizing peptide-metal ion interactions due to the smaller molecular size. Similar to the SPR results, the standard deviations were high (Table 2).

A high association rate (k_{on}) between a given peptide and immobilized nickel combined with a low dissociation rate (k_{off}) lead to a high affinity (low K_D). The peptide DLHSQNNY had the best affinity (lowest K_D) for nickel followed by the peptide THTAQETAK (Table 2). Interestingly, the two homologous peptides THTAQETAK and THTAEETAK (varying by one amino-acid residue highlighted in bold) showed different behaviors despite their very similar sequences (Figure 2) and displayed the highest and one of the lowest affinities among the synthetic peptides tested, respectively. A similar trend was observed in the SPR experiments where THTAQETAK showed affinity for Ni^{2+} while it was not possible to determine the affinity of THTAEETAK. As described by Elias et al. (2008), the affinity of peptides toward metal ions is also influenced by the charge of the peptides. A negatively charged peptide

can be electrostatically attracted to the positively charged Ni^{2+} . This was not observed in the present study for THTAEETAK and THTAQETAK, which have charges of -1 and 0 at neutral pH, respectively (Table 1).

The strategy using technologies of real-time molecule interaction analyses to screen for metal-chelating activity is a very new concept and the reports in the literature are few. However, in the present study, these designs were revealed to be very suitable for discriminating efficient MCPs from peptides with no chelating activity on the basis of slight differences in their amino acid sequences.

Dynamic measurements

By alternating electric potentials applied to the gold microelectrode, the negatively charged DNA nanolevers are either attracted to, or repelled from, the surface. This generates an oscillating orientation change of the DNA nanolevers, called switching. Due to the presence of the Cy5 probe, the position of the DNA nanolevers can be assessed relative to the gold surface. The intensity of the fluorescent light emitted by the Cy5 probe reports the distance to the gold surface due to a distance-dependent radiation-free energy transfer to the gold. In other words, the closer the Cy5 fluorophore is positioned to the quenching gold surface, the lower light it emits. The dynamic response (DR) characterizes the switching speed of DNA

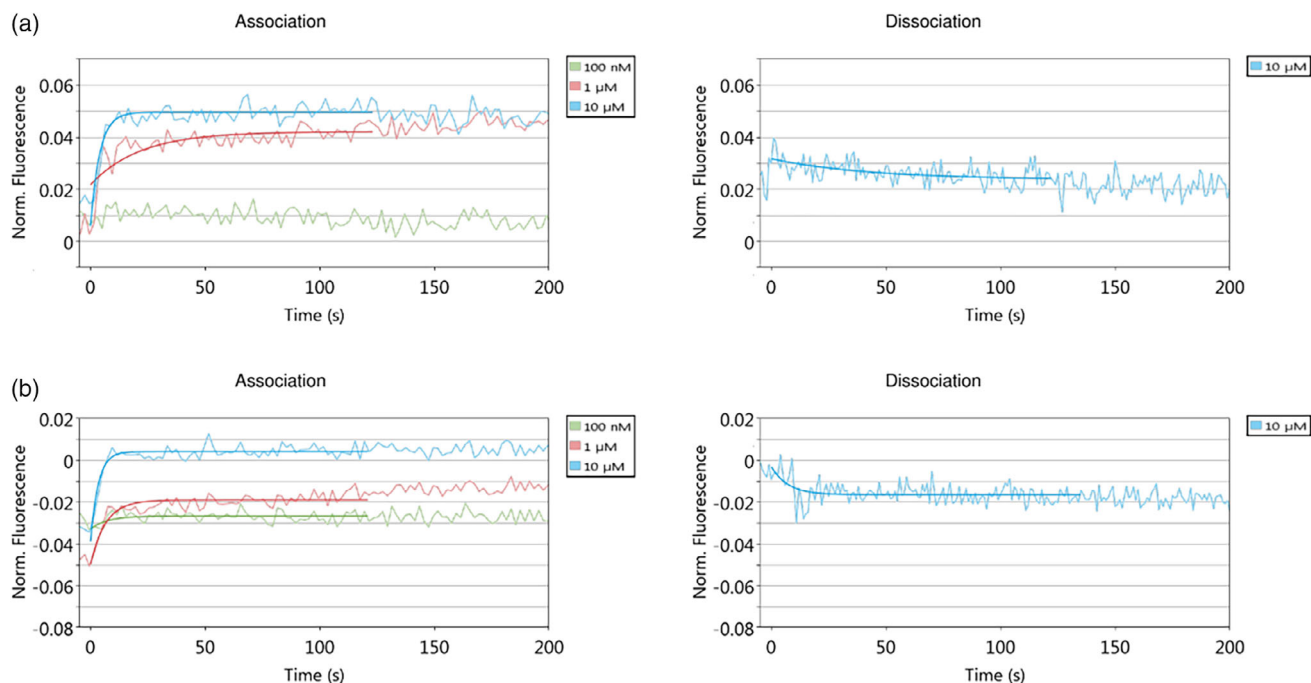


FIGURE 2 Association and dissociation data for (a) THTAQETAK and (b) THTAEETAK. Only one concentration is tested for dissociation since it is independent of analyte concentration.

nanolevers. When an analyte (e.g., peptide) binds to the immobilized Ni^{2+} , it induces a change in hydrodynamic friction and the switching speed slows down. High DR values indicate fast switching and vice versa. Note that large analytes affect the hydrodynamic friction more than smaller analytes, resulting in slower switching speed. Switching speed decreases upon analyte binding and increases upon dissociation. Note that DR is also used to detect unspecific binding. If ΔDR (difference of dynamic response measured in the absence, and in the presence of analytes) is not zero for E1 and E2 (reference electrode without Ni^{2+}), it could indicate unspecific binding between the peptide and the DNA nanolever.

The dynamic measurements are shown in Figure 3. All the investigated peptides had negligible effect on the motion of the nanolevers upon the voltage application ($|\Delta\text{DR}| < 2\%$) and no unspecific binding onto the DNA strand was detected. The bound peptides may be too small to elicit a significant change in DR as the dynamic measurements are sensitive to the size of the investigated analytes.

These results agree with those observed by El Hajj et al. (2021) for small synthetic peptides (2 to 6 residues). However, the authors also observed significant change in DR ($|\Delta\text{DR}|$ up to 3 to 10%) for soy protein hydrolysates in the sample spots and no unspecific binding. This indicates the presence of peptides in the soy protein hydrolysates that can bind Ni^{2+} and are larger than synthetic peptides investigated in our study.

Evaluating SPR and switchSENSE[®] as screening techniques for metal-chelating peptides

There are both similarities and differences between SPR and switchSENSE. These technologies both analyze the interaction between a tethered ligand (NTA- Ni^{2+}) and an analyte initially present in solution (peptide). They are both, to some extent, dependent on the size of the analytes. In SPR, the angle shift depends on the mass of the analyte, while in switchSENSE[®] larger molecules affect the hydrodynamic friction in dynamic measurements as well as the shielding effect in proximity sensing. Yet, a key difference between the two techniques is, that in switchSENSE[®], three Ni^{2+} are attached to a tri-NTA molecule per DNA nanolever while in SPR only one NTA molecule is tethered at a time. This provides three times as many potential binding spots and could also explain the lower K_D values obtained for switchSENSE[®] compared to SPR. Additionally, since the switchSENSE[®] requires much lower concentrations compared to the SPR, the sample amount requirement is lower. This also affects the solubility of the peptides and as such, the solubility issues experienced with SPR were not an issue in switchSENSE[®]. When using these techniques to screen for metal-chelating activity of peptides, it is important to keep in mind that when Ni^{2+} is bound to NTA, four of the six coordination sites on Ni^{2+} are occupied by the tetravalent NTA complexing agent. This is

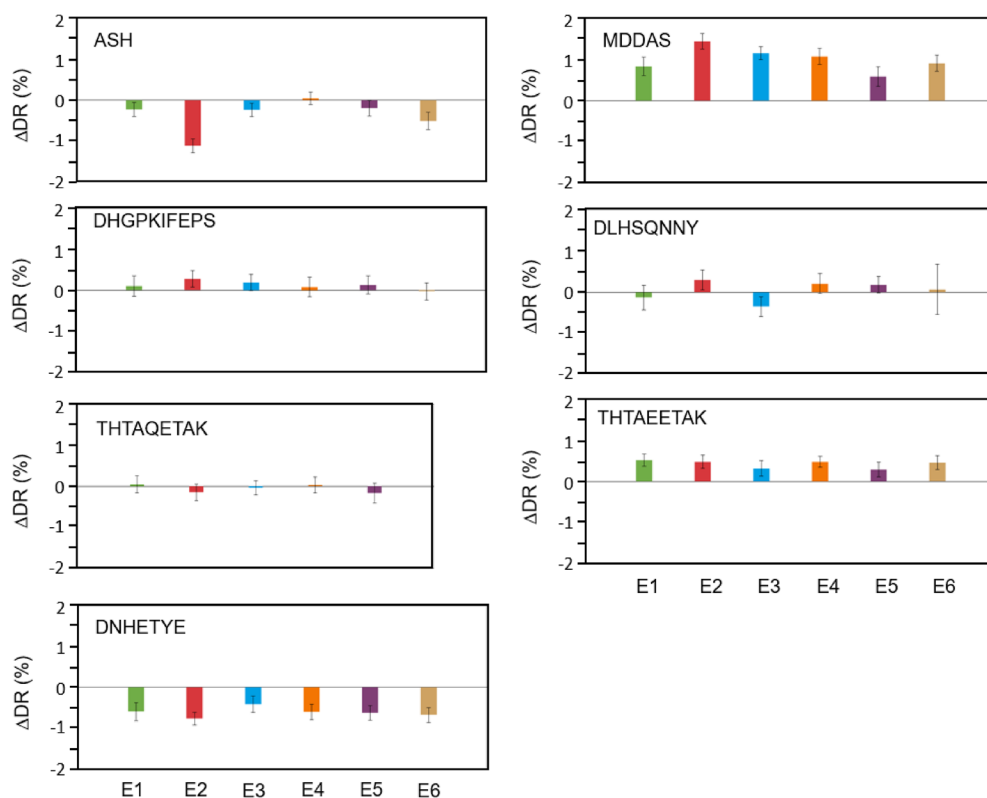


FIGURE 3 Molecular dynamics analysis in the presence of 1 μM synthetic peptides. The reference spots: E1 and E2 (without Ni^{2+}). The sample spots: E3–E6 (with immobilized Ni^{2+}). ΔDR : relative difference in dynamic response.

necessary to screen the Ni^{2+} binding using these techniques. This means that the peptide can only occupy the two remaining free sites on Ni^{2+} . If a peptide shows affinity for Ni^{2+} , it could potentially have an even higher affinity for free Ni^{2+} in solution where all six coordination sites are available. Moreover, when using peptides as chelators in solutions, like in food antioxidants, the way they bind to Ni^{2+} potentially changes. Depending on their sequence, length, and flexibility, peptides could bind to Ni^{2+} in ratios of 2:1 or 3:1. This means that, compared to EDTA which binds 1:1, higher concentrations of peptides might be necessary in food systems. The AnOxPePred chelation scores from Table 1 were plotted against the K_D from SPR and switchSENSE[®] (results not shown). Poor negative correlation ($R^2 = 0.281$) was observed between switchSENSE[®] and AnOxPePred while a moderate positive correlation ($R^2 = 0.6247$) was observed between SPR and AnOxPePred. Ideally, the correlation should be negative since a higher chelation score would translate to a higher affinity and thus a lower K_D . No correlation was observed when comparing K_D from SPR and switchSENSE[®] ($R^2 = 0.041$, $n = 5$).

CONCLUSION

In summary, this study aimed to investigate the potential of SPR and switchSENSE[®], two techniques typically utilized for larger molecules such as proteins and antibodies, to screen the affinity of peptides for immobilized Ni^{2+} and to compare these methods with the traditional ferrozine assay. Despite its widespread use, the ferrozine assay showed significant limitations when studying synthetic peptides. These include solubility-related challenges and significant variability, as indicated by high standard deviations. Dissolving peptides in DMSO alleviated the solubility issues. However, the use of DMSO increased the chelation% compared to water and could therefore overestimate the chelating activity of the investigated peptides. Additionally, of all the investigated peptides, only HHHHHH, a known Ni^{2+} chelating peptide used to tag proteins for affinity purification, exhibited iron chelation above 10%. These challenges emphasize the need for alternative methods for screening metal-chelating properties of peptides and complicate the direct comparison with results from SPR and switchSENSE[®].

In contrast, both SPR and switchSENSE[®] were capable of analyzing synthetic peptides effectively. SPR identified concentration-dependent binding to immobilized Ni^{2+} for five of the seven investigated peptides (ASH, DNHETYE, THTAQETAK, DLHSQNNY, DHGPKIFEPS), which indicates metal-chelating activity. However, peptide solubility was also an issue for SPR, and this should be addressed in future experiments to make the analysis more robust. On the other

hand, switchSENSE[®] differentiated between two peptides, THTAQETAK and THTAETAK, despite their similar sequences. Qualitatively, both SPR and switchSENSE[®] provided similar assessments of the two previous peptides in terms of binding/no binding, but it was not possible to measure the peptide with less affinity for Ni^{2+} , THTAETAK, using SPR.

Interestingly, no correlation was found between the K_D values determined by SPR and switchSENSE[®] for the investigated peptides. Given their respective advantages and limitations, this lack of correlation suggests that, rather than being direct alternatives to the ferrozine assay, SPR and switchSENSE[®] can act as complementary techniques for understanding peptide-metal ion interactions.

Looking forward, switchSENSE[®] shows promise as a screening technique for metal-chelating activity of peptides based on their amino acid sequences and provides an alternative to the solubility and consistency issues with analyzing peptides using the ferrozine assay. This is particularly significant considering these methods are normally used to study larger molecules. However, large standard deviations associated with K_D values obtained by both switchSENSE[®] and SPR underline the need for further optimization of these techniques. This sets the stage for future research focused on improving these methods for studying peptide-metal ion interaction and addressing the limitations encountered with the ferrozine assay.

AUTHOR CONTRIBUTIONS

Bjørliie, M., Jacobsen, C., Sørensen, A.M., Yesiltas, B., Canabady-Rochelle, L. conceived and designed the study. Bjørliie, M. wrote the first draft of the manuscript. Bjørliie, M., Irankunda, R. carried out the research. Bjørliie, M., Girardet, J. created graphics. Bjørliie, M., Irankunda, R., Girardet, J., Canabady-Rochelle, L. analyzed the data. All authors contributed to revising and approved the final draft of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

No human or animal subjects were used in the research for this study.

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REFERENCES

- Asioli D, Aschemann-Witzel J, Caputo V, Vecchio R, Annunziata A, Næs T, et al. Making sense of the “clean label” trends: a review of consumer food choice behavior and discussion of industry implications. *Food Res Int.* 2017;99:58–71. <https://doi.org/10.1016/j.foodres.2017.07.022>
- Bornhorst JA, Falke JJ. Purification of proteins using polyhistidine affinity tags. *Methods Enzymol.* 2000;326:245. [https://doi.org/10.1016/S0076-6879\(00\)26058-8](https://doi.org/10.1016/S0076-6879(00)26058-8)
- Canabady-Rochelle LLS, Selmeçzi K, Collin S, Pasc A, Muhr L, Boschi-Muller S. SPR screening of metal chelating peptides in a hydrolysate for their antioxidant properties. *Food Chem.* 2018; 239:478–85. <https://doi.org/10.1016/j.foodchem.2017.06.116>
- Decker E, Bayram I. Why does lipid oxidation in foods continue to be such a challenge? In: Proceedings of the Virtual 2021 AOCS Annual Meeting & Expo. 2021 <https://doi.org/10.21748/am21.39>
- El Hajj S, Irankunda R, Camaño Echavarría JA, Arnoux P, Paris C, Stefan L, et al. Metal-chelating activity of soy and pea protein hydrolysates obtained after different enzymatic treatments from protein isolates. *Food Chem.* 2023;405:134788. <https://doi.org/10.1016/j.foodchem.2022.134788>
- El Hajj S, Sepúlveda Rincón CT, Girardet JM, Cakir-Kiefer C, Stefan L, Zapata Montoya JE, et al. Electrically switchable nanolever technology for the screening of metal-chelating peptides in hydrolysates. *J Agric Food Chem.* 2021;69(31):8819–27. <https://doi.org/10.1021/acs.jafc.1c02199>
- Elias RJ, Kellerby SS, Decker EA. Antioxidant activity of proteins and peptides. *Crit Rev Food Sci Nutr.* 2008;48(5):430–41. <https://doi.org/10.1080/10408390701425615>
- Farvin S, Jacobsen C. Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. *Food Chem.* 2013;138(2–3):1670–81. <https://doi.org/10.1016/j.FOODCHEM.2012.10.078>
- Farvin SKH, Baron CP, Nielsen NS, Jacobsen C. Antioxidant activity of yoghurt peptides: part 1-in vitro assays and evaluation in ω -3 enriched milk. *Food Chem.* 2010;123(4):1081–9. <https://doi.org/10.1016/j.foodchem.2010.05.067>
- Frankel EN. Lipid oxidation. Cambridge, UK: Woodhead Publishing Limited; 2005. <https://doi.org/10.1533/9780857097927>
- Hamit CS, Nam KC, Abeyratne EDNS. Bioactive peptide production and determination of functional properties using crude water extracts of *Ulva lactuca* (Chlorophyta) and *Sargassum crassifolium* (Phaeophyceae) collected from Sri Lanka. *Appl Phycol.* 2022;3(1):73–82. <https://doi.org/10.1080/26388081.2022.2063758>
- Hermund DB, Heung SY, Thomsen BR, Akoh CC, Jacobsen C. Improving oxidative stability of skin-care emulsions with antioxidant extracts from brown alga *Fucus vesiculosus*. *J Am Oil Chem Soc.* 2018;95(12):1509–20. <https://doi.org/10.1002/aocs.12156>
- Irakunda R, Bjørle M, Yesiltas B, Muhr L, Canabady-Rochelle L, Jacobsen C. Evaluation of primary and secondary oxidation products in fish oil-in-water emulsions: effect of metal-complexing peptides and protein hydrolysates. *Food Chem.* 2024;439:138042. <https://doi.org/10.1016/J.FOODCHEM.2023.138042>
- Jacobsen C. Oxidation mechanisms in fish oil enriched emulsions. Lyngby, Denmark: Technical University of Denmark; 1999.
- Knecht S, Ricklin D, Eberle AN, Ernst B. Oligohis-tags: mechanisms of binding to Ni²⁺-NTA surfaces. *J Mol Recognit.* 2009;22(4): 270–9. <https://doi.org/10.1002/jmr.941>
- Lv Y, Liu Q, Bao X, Tang W, Yang B, Guo S. Identification and characteristics of iron-chelating peptides from soybean protein hydrolysates using IMAC-Fe³⁺. *J Agric Food Chem.* 2009; 57(11):4593–7. <https://doi.org/10.1021/jf9000204>
- Martell A, Smith R, Motekaitis R. NIST standard reference database 46: critically selected stability constants of metal complexes. 2001.
- McClements DJ, Decker EA. Lipids. Fennema's food chemistry. 5th ed. Boca Raton, FL: CRC Press; 2017. <https://doi.org/10.1201/9781315372914>
- Muhr L, Pontvianne S, Selmeçzi K, Paris C, Boschi-Muller S, Canabady-Rochelle L. Chromatographic separation simulation of metal-chelating peptides from surface plasmon resonance binding parameters. *J Sep Sci.* 2020;43(11):2031–41. <https://doi.org/10.1002/JSSC.201900882>
- Narmuratova Z, Hentati F, Girardet JM, Narmuratova M, Cakir-Kiefer C. Equine lactoferrin: antioxidant properties related to divalent metal chelation. *LWT.* 2022;161:113426. <https://doi.org/10.1016/J.LWT.2022.113426>
- Olsen TH, Yesiltas B, Marin FI, Pertseva M, García-Moreno PJ, Gregersen S, et al. AnOxPePred: using deep learning for the prediction of antioxidative properties of peptides. *Sci Rep.* 2020; 10(1):21471. <https://doi.org/10.1038/s41598-020-78319-w>
- Sakiyama H. Predicting conformers of flexible metal complexes using deep neural network. Computational and data-driven chemistry using artificial intelligence: fundamentals, methods and applications. Amsterdam, Netherlands: Elsevier; 2022. p. 193–216. <https://doi.org/10.1016/B978-0-12-822249-2.00007-4>
- Vavrusova M, Pindstrup H, Johansen LB, Andersen ML, Andersen HJ, Skibsted LH. Characterisation of a whey protein hydrolysate as antioxidant. *Int Dairy J.* 2015;47:86–93. <https://doi.org/10.1016/j.idairyj.2015.02.012>
- Xiao CQ, Huang Q, Zhang Y, Zhang HQ, Lai L. Binding thermodynamics of divalent metal ions to several biological buffers. *Thermochim Acta.* 2020;691:178721. <https://doi.org/10.1016/J.TCA.2020.178721>
- Yesiltas B, García-Moreno PJ, Gregersen S, Olsen TH, Jones NC, Hoffmann SV, et al. Antioxidant peptides derived from potato, seaweed, microbial and spinach proteins: oxidative stability of 5% fish oil-in-water emulsions. *Food Chem.* 2022;385:132699. <https://doi.org/10.1016/j.foodchem.2022.132699>
- Zhang J, Chen X, Yang R, Ma Q, Qi W, Sanidad KZ, et al. Thermally processed oil exaggerates colonic inflammation and colitis-associated colon tumorigenesis in mice. *Cancer Prev Res.* 2019;12(11):741–50. <https://doi.org/10.1158/1940-6207.CAPR-19-0226>

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