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**Acrylamide mitigation using zein–polysaccharide complex particles**

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**Abstract**

Protein–polysaccharide biopolymer particles composed of potential acrylamide inhibitors, including zein, chitosan, alginate, and pectin, were used for acrylamide mitigation in this study. The synergistic effect of zein and polysaccharides in complex particles was hypothesized to inhibit acrylamide formation at high temperature. A reaction model utilizing a glucose and asparagine solution heated at 170 ◦C for 30 min in a heating block was implemented. Zein, zein–polysaccharide complex particles, and a mixture of zein and polysaccharides (non- complex particles) were used as acrylamide inhibitor solutions. The highest acrylamide reduction was observed at a zein concentration of 0.67% (w/v). Because glutamine and asparagine are the major amino acids found in zein, higher zein concentration (>1%, w/v) increased acrylamide formation. An increase in charge or stability was noted after particle formation, such as zein–sodium alginate (–49.07 to –57.20 mV) and zein–pectin (–27.80 to –31.57 mV), coupled with an increase in average size. Zein–polysaccharide complex particles successfully reduced acrylamide concentration in a heating block model. The most effective acrylamide inhibitor was zein–alginate (1:0.50). However, zein–chitosan particles exerted the opposite effect, thereby promoting acrylamide formation. The acrylamide content in fried potato strips dipped in complex particles (11.07–13.22 μg/mL) was lower than that observed in control (16.08 μg/mL). The interfacial rheology of sticky adsorbing particles with food surfaces requires further investigation.

## 1. Introduction

Acrylamide has been classified as a group 2A carcinogen (possibly carcinogenic to humans) by the International Agency for Research on Cancer. Animal studies have also shown that acrylamide is a mutagen in humans. It presents in heat-treated starchy foods (>120 ◦C) and is formed during the Maillard reaction (Krishnakumar & Visvanathan, 2014). Acrylamide is formed via Strecker degradation involving sugar-derived carbonyl compounds and asparagine-yielded aldehyde with the α-carbon of amino acids. Subsequently, carbonyl compounds, amino acids, and the degraded derivatives produce the desired flavored compounds and melanoidin pigments (Claeys, de Vleeschouwer, & Hendrickx, 2005; Mottram, Wedzicha, & Dodson, 2002). As acrylamide can be detoxified in the body, the risk associated with its consumption through foods is considered low. However, an overload of arylamide that exceeds the detoxification capacity of the human body can put consumers at a higher risk. Therefore, numerous research attempts are being made to develop strategies for reducing acrylamide formation in foods.

Ingredient substitution, such as the replacement of reducing sugar (e. g., fructose and glucose) with sucrose, was found to reduce acrylamide formation; however, the development of brown color via the Maillard reaction was also reduced (Anese et al., 2009). Mono- and divalent cations (e.g., $Na^{+}$and $Ca^{2+}$) prevented the formation of Schiff base, which is an intermediate for acrylamide formation (Gokmen & Senyuva, 2007). Although studies have proposed that the addition of antioxidants

prevents acrylamide formation, results have been inconsistent. Phenolic compounds in virgin olive oil, flavonoids (e.g., naringenin) and anti-oxidants in bamboo leaf extract have been reported to effectively reduce acrylamide formation (Cheng, Zeng, et al., 2009; Napolitano, Morales, Sacchi, & Fogliano, 2008; Zhang, Chen, Zhang, Wu, & Zhang, 2007). In contrast, evidence has demonstrated that butyrate hydroxyl toluene, sesamol, curcumin, vitamin E, and dragon fruit extract increase acrylamide formation in fried potato (Cheng, Zeng, et al., 2009). Hydrocolloid is another promising ingredient used to inhibit acryl-amide formation. Zeng et al. (2010) used eight hydrocolloids to reduce the formation of acrylamide; they found that alginic acid and pectin were the most effective acrylamide inhibitors. Chitosan is also an acrylamide inhibitor because its free amino groups can compete with asparagine in binding with the reducing sugar. The molecular weight (MW) and degree of deacetylation of chitosan exert an effect on acryl-amide reduction. Low MW (50–190 kDa) and high degree of deacetylation (86.5–92.8%) of chitosan could reduce the formation of acrylamide by ≤81% (Chang, Sung, & Chen, 2016; Sansano et al., 2016, 2017; Sung, Chang, Chou, & Hsiao, 2018). Furthermore, the addition of amino acids or protein base ingredients could either increase or decrease acrylamide content. The amino acids that were the most effective in acrylamide reduction were glycine (Anese et al., 2009; Brathen, Kita, Knutsen, & Wicklund, 2005), cysteine, and lysine (Claeys et al., 2005). Their mechanism of action is mainly based on the competition between free amino and asparagine to bind with the reducing sugar. However, cysteine presents an unpleasant odor which limits its application in foods (Claeys et al., 2005). In contrast, the addition of protein-rich food (lean fish meat) in potato has been shown to increase acrylamide levels (Rydberg et al., 2003).

Zein is a hydrophobic, insoluble, and prolamin-rich corn protein. The production of zein nanoparticle is often achieved by antisolvent precipitation with ethanol. Zein particles have poor dispersibility and interfacial functionality; thus, there was an effort to use zein with polysaccharides, such as chitosan (Li, Xu, et al., 2018; Wang et al., 2015, 2016, 2018), alginate (Hu & McClements, 2015), alginate, and/or so-dium caseinate (Feng & Lee, 2016; Li, Xu, et al., 2018; Patel, Bouwens, & Velikov, 2010). The zein–polysaccharide biopolymer particles have been used to stabilize and emulsify in Pickering emulsions mostly for encapsulation and controlled release purposes (Li, Xu, et al., 2018). The present study investigated the use of zein–polysaccharide complex particles to reduce acrylamide formation. The complex particles were composed of previously reported potential acrylamide inhibitors such as chitosan, alginate, and pectin. It was hypothesized that the synergistic effect of zein and polysaccharides on the formation of protein–polysaccharide complexes enhance acrylamide inhibition at high temperature more effectively than that by zein or polysaccharides alone. The facile method was used to generate particles via antisolvent precipitation using electrostatic force for stabilization between protein and anionic polysaccharides. Two models (chemical and food) were used to compare the mitigation effects of zein–polysaccharide complex particles, zein, polysaccharides, and the mixture of zein and polysaccharides (non-complex particles) on acrylamide formation.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Acrylamide (≥99%), glucose, low MW chitosan (CAS no 9012-76-4, 50,000–190,000 Da, viscosity 20–300 cps), acetic acid, pectin from citrus peel (CAS no 9000-69-5, galacturonic acid ≥74.0%), zein from maize (CAS no 9010-66-6), zinc acetate dihydrate, potassium hexacyanoferrate (II) trihydrate, and formic acid were purchased from Sigma–Aldrich (St. Louis, MI, USA.). Asparagine was obtained from ACROS Organics (Morris Plains, NJ, USA). Sodium alginate (CAS no 9005-38-3, viscosity 5–40 cps), hydrochloric acid, sodium hydroxide, ethanol, rhodamine B, acridine orange, fluoresceinamine, and ruthenium red were purchased from Fisher Scientific (Waltham, MA, USA.). Milli-Q water was used

for the purpose of experimentation. Potatoes and sunflower oil were purchased from the supermarket.

2.2. Solution preparation

2.2.1. Zein solution

Zein concentrations (0.5, 1.0, 2.0, 3.0, 4.0, 5.0% weight/volume [w/ v]) were prepared by dissolving zein in 80% ethanol (100 mL) and stirring for 3 h. Zein solution (1 mL, pH 5.8) was mixed with 0.5 M glucose solution (1 mL) and 0.5 M asparagine solution (1 mL) to obtain final zein concentrations of 0.17, 0.30, 0.67, 1.00, 1.33, and 1.67% (w/ v).

2.2.2. Chitosan solution

Low MW chitosan (1.0 g) was solubilized in 1% acetic acid (100 mL), and the solution was stirred overnight for complete dissolution. Chitosan solution was diluted to 0.6% and 0.3% (vol/vol [v/v]) with 1% acetic acid, and the pH was adjusted to 4.0 using 0.1 M and 1.0 M NaOH.

2.2.3. Sodium alginate solution

Sodium alginate (1.0 g) was dispersed in Milli-Q water (100 mL), heated at 70 ◦C with stirring for 30 min, and cooled down to ambient temperature using tap water. The solution was diluted to 0.6% and 0.3% (v/v), and the pH was adjusted to 4.0 using 0.1 M and 1 M HCl.

2.2.4. Pectin solution

Pectin from citrus peel (1.0 g) was added to Milli-Q water (100 mL), and the solution was stirred overnight. Pectin solution was diluted to 0.6% and 0.3% (v/v) using Milli-Q water, and the pH was adjusted to 4.0 using 0.1 M and 1 M NaOH.

2.3. Formation of zein–polysaccharide complex particles

Zein solution (2%) was prepared as described in section 2.2.1. Subsequently, the solution was mixed with chitosan, sodium alginate, and pectin in three ratios, namely 1:0.50, 1:0.30, and 1:0.15 (w/w). After vortexing for 1 min, all suspensions were placed in a Genevac-EZ 2 plus (Warminster, PA, USA.) for 2 h so that ethanol could evaporate and were then centrifuged. The temperature was maintained at 40 ◦C under the low-pressure mode. The complex particles in the supernatant were collected and stored at 4 ◦C for further analysis. The complexation method was performed using a modified protocol of a previously described method (Li, Xu, et al., 2018). The mixture of zein and poly-saccharides (non-complex particles) was prepared by direct mixing of the zein solution with the polysaccharide solution in three ratios (as previously described) and vortexing for 1 min.

2.4. Acrylamide inhibition in a chemical model

The reaction in the chemical model was performed according to the method reported by Gokmen and Senyuva (2007). The reaction mixture contained 0.5 M glucose solution (1 mL), 0.5 M asparagine solution (1 mL), and acrylamide inhibitors (1 mL) in a tube with a screw cap. Acrylamide inhibitor contained zein solutions (0.17, 0.30, 0.67, 1.33, 1.67% w/v), zein–polysaccharide complex particle solutions (1:0.15, 1:0.3, 1:0.50), and zein–polysaccharide non-complex particles (1:0.15, 1:0.3, 1:0.50). All samples were heated at 170 ◦C for 30 min in a heating block (VWR Analog Heat Block; VWR, Radnor, PA, USA). The temper-ature fluctuation was maintained within ±2 ◦C. The heated samples were immediately cooled in an ice bath for 5 min.

2.5. Acrylamide inhibition in a food model

Potatoes were washed, peeled, and cut into strips (1 cm ×1 cm ×5 cm). These strips (50 g) were soaked in 50 mL of zein solution (2.0% w/ v), zein–chitosan (1:0.50), zein–alginate (1:0.50), or zein–pectin (1:0.50) complex particle solutions for 30 min, drained for 2 min, and fried at 170 ◦C for 3 min using a deep fat fryer (Cookworks™, Milton Keynes, UK). A sample not containing protein–polysaccharide was used as control. After frying, all samples were cooled down and drained using a sieve. The process was performed using the modified method described by Zeng et al. (2010) with minor modification. Acrylamide inhibition (%) was calculated using the equation mentioned below.

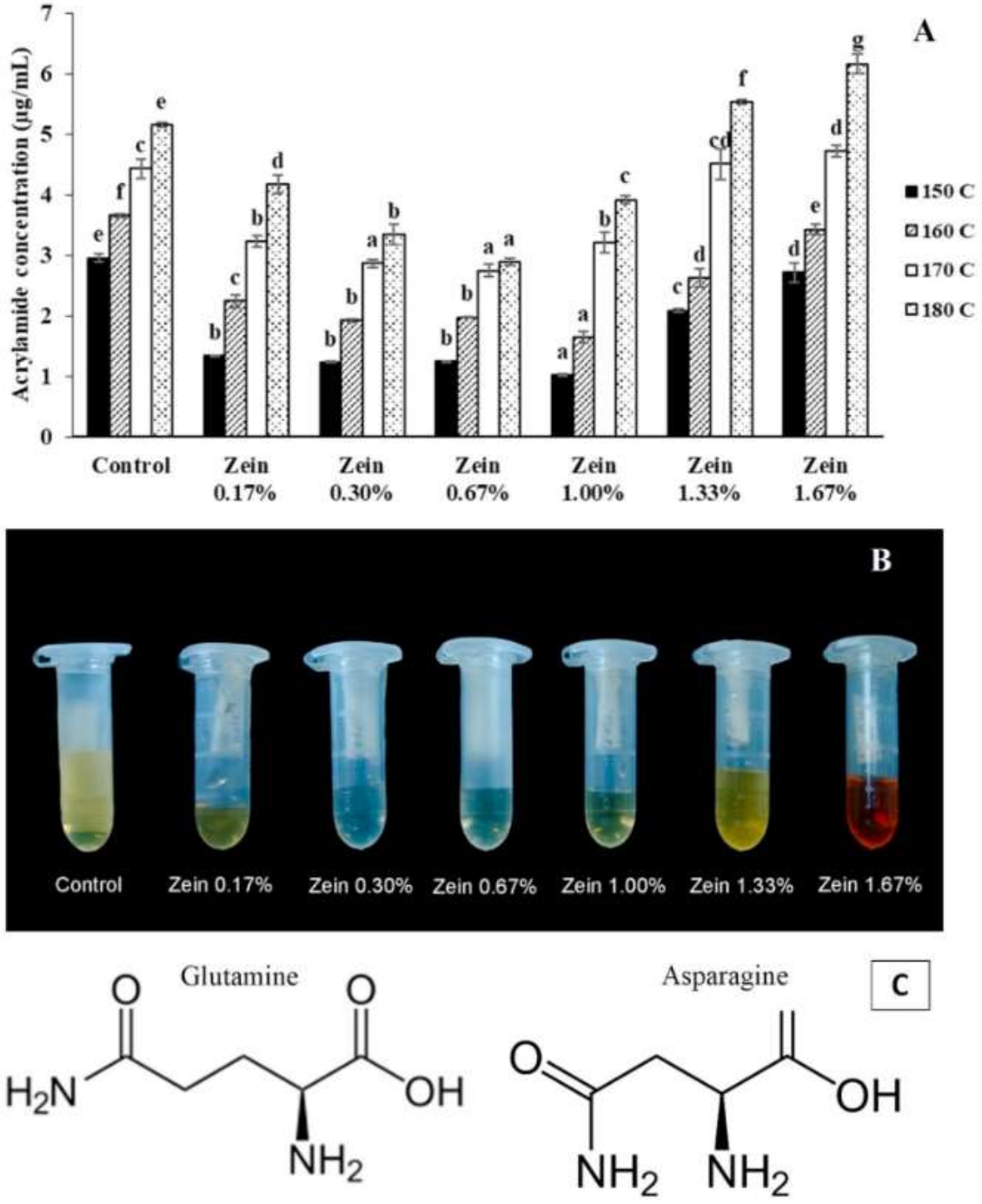


Fig. 1.(A) The dose-dependent inhibitory effects of zein solutions heated in a heating block at 150 ◦C, 160 ◦C, 170 ◦C, and 180 ◦C for 30 min on acrylamide; (B) browning formation at various concentrations of zein heated in a heating block at 170 ◦C for 30 min; (C) structure of glutamine and asparagine. $^{a-g}$ mean ± standard deviation with different lowercase letters in superscript indicating significant difference ($p \leq 0.05$) at the same temperature.

$$\% \, Acrylamide \, inhibition = \frac{Acrylamide \, content \, control - Acrylamide \, content \, sample}{Acrylamide \, content \, control} \times 100$$

2.6. Acrylamide determination

2.6.1. Preparation of acrylamide standard curve

The stock solution of acrylamide standard (1 mg/mL) was prepared and diluted to a series of concentrations (1–100 µg/mL). A calibration curve was constructed using the peak area of acrylamide eluted from the column against the concentration. Acrylamide concentration was calculated based on the solution concentration and the injection volume. In case an unidentified peak was suspected to be acrylamide, the sample was spiked with acrylamide standard for confirmation. The acrylamide standard equation obtained was as follows: y =63.223x +36.509, $R^2$ =0.9991 (data not shown).

2.6.2. Determination of acrylamide in a chemical model

After heating, all sample solutions were diluted (1:10) with Milli-Q water, filtered using a nylon filter (0.45 µm), and transferred to a vial for chromatographic analysis. Acrylamide was analyzed in triplicate using a slightly modified protocol of the method described by Galani, Patel, and Talati (2017). Using an auto sampler, sample solution (2 µL) was injected in the high-performance liquid chromatography (HPLC) device equipped with a diode array detector and a Zorbax 300 extend-C18 analytical column (2.1 ×100 mm, 3.5 µm) (1200 Series system, Agilent, Santa Clara, CA, USA). Formic acid solution (0.1% in water) was used as a mobile phase at a flow rate of 0.1 mL/min, running time of 5 min, and retention time of 3.4 min (for acrylamide). The chromatogram of acrylamide was recorded at 210 nm.

2.6.3. Determination of acrylamide in a food model

Grounded sample (1 g) was placed in a centrifuge tube. Carrez I, II (500 µL each) and 1.2% acetic acid (9 mL) were added, and the solution was mixed vigorously for 2 min. Carrez I was prepared by dissolving zinc acetate dihydrate (21.9 g) with acetic acid (3.0 g) in Milli-Q water and adjusting the volume to 100 mL. Carrez II was prepared by dissolving potassium hexacyanoferrate (II) trihydrate (10.6 g) in Milli-Q water and adjusting the volume to 100 mL. After mixing, sample (1 mL) was transferred to an Eppendorf tube using a 1 mL syringe. Centrifugation (Centrifuge 5424R; Eppendorf, Hamburg, Germany) was performed at 10,000 rpm for 10 min at 0 ◦C. The supernatant was collected and diluted (1:10) with Milli-Q water prior to HPLC analysis. Acrylamide extraction was performed using a modified protocol of the method described by Gokmen and Senyuva (2007).

2.7. Characterizationof zein–polysaccharide complex particles

2.7.1. Particle size and charge measurement

Measurement of particle size distribution of a suspension with relatively small particles was conducted using Dynamic Light Scattering (Malvern Nanoseries Instruments, Worcestershire, UK). Samples were diluted with Milli-Q water (1:20) prior to analysis. The Z-average diameter and polydispersity index (PDI) were calculated from the measured distributions. The zeta potential of the particles in the suspensions was determined using an Electrophoretic Light Scattering device (Malvern Nanoseries Instruments). Samples were diluted with pH- adjusted water (pH 4.0) prior to measurement to avoid multiple scattering effects.

2.7.2. Confocal laser scanning microscopy

Confocal laser scanning microscopy was carried out using LSM 900 upright with Airyscan 2 device (Carl Zeiss, Oberkochen, Germany). Zein–chitosan complex particles were dyed with a blend of fluorescent colorant solutions. Chitosan and zein were colored with rhodamine B and acridine orange, respectively. The dyed samples were placed on slides with coverslips ≥3 h before visualization to prevent particle mobility. Confocal laser scanning images were captured by selective emission at 271/520 nm for rhodamine B, 543/565 nm for acridine orange, 490/520 nm for fluoresceinamine, and 398/488 nm for ruthenium red (Galas et al., 2018; Nieuwland, Papen-Bottenhuis, Drost, Slaghek, & Erich, 2016).

2.8. Statistical analysis

The data were analyzed using analysis of variance and the least significance difference test to determine differences between means (significance level: 0.05) using the SPSS software package version 16 (IBM company, Chicago, IL, USA.). All experiments and measurements were performed in triplicate.

## 3. Results and discussion

3.1. Effect of zein on acrylamide inhibition

The inhibitory effect of zein on acrylamide was exerted in a dose- and temperature-dependent manner. The lowest and highest concentrations of zein which resulted in a substantial reduction of acrylamide formation were 0.17% (w/v) and 0.67% (w/v), respectively (Fig. 1A). Zein contains hydrophobic amino acids, such as proline, glutamine, and asparagine (Elzoghby, Samy, & Elgindy, 2012). The acrylamide content was indicated by brown color formed after heating (Fig. 1B). It has also been reported that acrylamide is generated from the Maillard reaction during heat treatment of amino acids and reducing sugar (Mottram et al., 2002).

However, the use of high concentrations of zein (>1%, w/v) exerted the opposite effect, resulting in higher acrylamide formation (1A). Glutamine (17.9–19.5%) and asparagine (4.6–5.2%) are the major amino acids found in zein (Gasteiger et al., 2005). High amounts of these amino acids increased acrylamide formation. Reaction of glutamine with equimolar amounts of glucose at 180 ◦C was found to rapidly in-crease acrylamide formation (Stadler et al., 2002) as the structure of glutamine is similar to that of asparagine (Claeys et al., 2005) (Fig. 1C). The addition of glutamine promoted acrylamide formation kinetics which studied in an asparagine-glucose model system (0.01 M, pH 6.0), and the solution was heated to 140◦C–200 ◦C (Claeys et al., 2005). However, reduction in acrylamide formation was reported after adding a small amount of glutamine (35 mM) in a food model (homogenized potato) and heating the solution in an oven at 180 ◦C for 25 min. Glutamine and glycine caused the highest reduction in the formation of acrylamide (76%), followed by lysine (43%), and alanine (14%) (Rydberg et al., 2003).

Moreover, it has been reported that environmental factors, especially the pH, also affect acrylamide mitigation (Rydberg et al., 2003). At pH 8.0, the α-amino group of asparagine exhibited a low pKa (8.9 at 25 ◦C), limiting its ability to protonate compared with other amino acids. Therefore, it is more likely that asparagine reacts with the aldehyde groups of carbohydrates, resulting in the formation of Schiff base, which is the initial step in the process of acrylamide formation. The pH of the zein solution used in our study was 5.3–5.6 as it has been reported that acrylamide was rapidly degraded under acidic condition (Rydberg et al., 2003).

Table 1 Average particle size, polydispersity index (PDI), and zeta potential of zein, polysaccharides, zein–polysaccharide complex particles, and zein and polysaccharides non-complex particles.

| Sample | Z-average (nm) | PDI | Zeta potential (mV) |
|---|---|---|---|
| Zein 2.0% | 323.76 ± 32.67 | 0.32 ± 0.04 | 25.93 ± 0.25 |
| **Polysaccharides** | | | |
| CS 1.0% | 1001.30 ± 69.04$^{e}$ | 0.54 ± 0.15$^{a}$ | 48.10 ± 0.66$^{h}$ |
| CS 0.6% | 671.80 ± 43.68$^{d}$ | 0.53 ± 0.14$^{a}$ | 47.33 ± 0.91$^{h}$ |
| CS 0.3% | 413.37 ± 27.66$^{ab}$ | 0.47 ± 0.07$^{a}$ | 44.07 ± 0.58$^{g}$ |
| Alg 1.0% | 490.20 ± 22.21$^{bc}$ | 0.31 ± 0.02$^{a}$ | −47.33 ± 1.56$^{a}$ |
| Alg 0.6% | 520.30 ± 81.18$^{c}$ | 0.30 ± 0.02$^{a}$ | −43.87 ± 1.90$^{b}$ |
| Alg 0.3% | 403.37 ± 10.90$^{a}$ | 0.40 ± 0.03$^{a}$ | −40.20 ± 1.97$^{c}$ |
| Pec 1.0% | 388.27 ± 15.17$^{a}$ | 0.30 ± 0.02$^{a}$ | −30.90 ± 1.14$^{d}$ |
| Pec 0.6% | 414.57 ± 33.47$^{ab}$ | 0.25 ± 0.02$^{a}$ | −26.23 ± 0.35$^{e}$ |
| Pec 0.3% | 442.50 ± 48.77$^{abc}$ | 0.22 ± 0.01$^{a}$ | −22.87 ± 0.81$^{f}$ |
| **Zein–polysaccharide complex particles** | | | |
| Z:CS 1:0.50 | 2251.33 ± 26.10$^{c}$ | 0.17 ± 0.04$^{aba}$ | 61.30 ± 2.10$^{ga}$ |
| Z:CS 1:0.30 | 1808.33 ± 5.69$^{c}$ | 0.23 ± 0.02$^{abca}$ | 56.20 ± 0.17$^{fa}$ |
| Z:CS 1:0.15 | 1181.33 ± 4.86$^{ba}$ | 0.13 ± 0.06$^{aa}$ | 48.53 ± 0.87$^{e}$ |
| Z:Alg 1:0.50 | 729.70 ± 7.63$^{aba}$ | 0.33 ± 0.03$^{c}$ | −57.20 ± 0.44$^{aa}$ |
| Z:Alg 1:0.30 | 706.73 ± 13.21$^{aa}$ | 0.33 ± 0.03$^{ca}$ | −56.57 ± 0.21$^{aa}$ |
| Z:Alg 1:0.15 | 649.67 ± 12.91$^{ba}$ | 0.28 ± 0.01$^{bca}$ | −49.07 ± 0.57$^{ba}$ |
| Z:Pec 1:0.50 | 592.43 ± 9.33$^{a}$ | 0.19 ± 0.03$^{ab}$ | −31.57 ± 0.31$^{ca}$ |
| Z:Pec 1:0.30 | 483.97 ± 3.12$^{a}$ | 0.16 ± 0.02$^{aba}$ | −30.20 ± 0.27$^{ca}$ |
| Z:Pec 1:0.15 | 401.73 ± 15.43$^{aa}$ | 0.17 ± 0.01$^{aba}$ | −27.80 ± 0.35$^{da}$ |
| **Zein and polysaccharides (non-complex particles)** | | | |
| Z:CS 1:0.50 | 2334.00 ± 9.54$^{c}$ | 0.49 ± 0.35$^{ab}$ | 47.70 ± 0.36$^{g}$ |
| Z:CS 1:0.30 | 1855.66 ± 27.10$^{bc}$ | 0.54 ± 0.36$^{c}$ | 45.07 ± 0.06$^{f}$ |
| Z:CS 1:0.15 | 1565.33 ± 31.66$^{b}$ | 0.47 ± 0.11$^{ab}$ | 43.17 ± 0.76$^{e}$ |
| Z:Alg 1:0.50 | 646.33 ± 63.24$^{a}$ | 0.33 ± 0.17$^{ab}$ | −46.50 ± 1.35$^{a}$ |
| Z:Alg 1:0.30 | 658.63 ± 51.20$^{a}$ | 0.42 ± 0.09$^{ab}$ | −46.77 ± 1.00$^{a}$ |
| Z:Alg 1:0.15 | 527.50 ± 29.70$^{a}$ | 0.47 ± 0.07$^{ab}$ | −42.80 ± 0.92$^{b}$ |
| Z:Pec 1:0.50 | 574.57 ± 33.81$^{a}$ | 0.21 ± 0.03$^{a}$ | −26.50 ± 0.40$^{c}$ |
| Z:Pec 1:0.30 | 496.47 ± 36.63$^{a}$ | 0.24 ± 0.02$^{a}$ | −25.53 ± 0.06$^{c}$ |
| Z:Pec 1:0.15 | 440.00 ± 17.16$^{a}$ | 0.26 ± 0.03$^{a}$ | −22.03 ± 0.45$^{d}$ |

Abbreviations: Alg, alginate; CS, chitosan; Pec, pectin; Z, zein. $^{a–h}$ mean ±SD with different lowercase letters in superscripts indicates a significant difference ($p \leq 0.05$) in polysaccharides, zein–polysaccharide complex particles, and non-complex particles. $^{a}$ Indicates a significant difference ($p \leq 0.05$) using t-test between complex particles and non-complex particles.

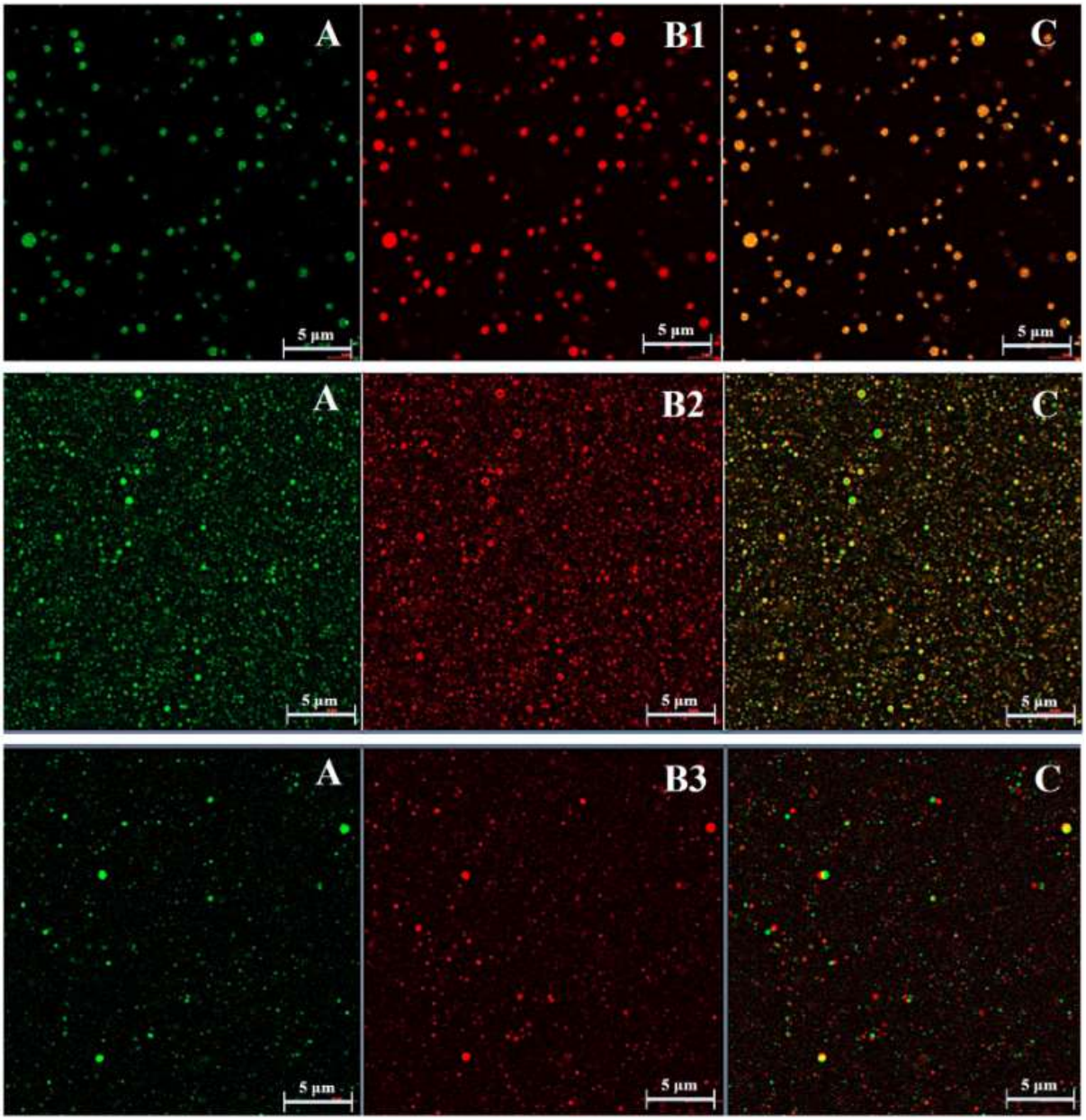


Fig. 2. Confocal laser scanning microscope (CLSM) images (63 ×) of zein–chitosan (top), zein–alginate (middle), and zein–pectin complex particles (bottom). The fluorescent dye for zein (A) was acridine orange, which showed emission at 543/565 nm; that for chitosan (B1) was rhodamine B, which showed emission at 271/ 520 nm; that for sodium alginate (B2) was fluoresceinamine, which showed emission at 490/520 nm; that for pectin (B3) was ruthenium red, which showed emission at 398/488 nm, and (C) the intersection between A and B showed particles complexed between zein and each polysaccharide. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

### 3.2. Effect of zein–polysaccharide complex particles on acrylamide inhibition

#### 3.2.1. Formation and characterization of zein–polysaccharide complex particles

Zein was used to form complexes with chitosan, sodium alginate, and pectin. The colloidal zein–polysaccharide complex particle was prepared using the antisolvent precipitation method. This was achieved by mixing zein in ethanol solution (80%, v/v) with ionic polysaccharide solution (in water) and stirring at ambient temperature. Zein is miscible with an aqueous ethanol phase; however, water is a poor solvent for the dissolution of zein. Thus, the zein ethanol solution is sheared into the water phase and forms small droplets caused by the excellent miscibility of ethanol and water (Wang et al.,

2016). Zein–polysaccharide particles were precipitated after decreasing the concentration of ethanol in the dispersed phase through evaporation. The zeta potential is used to estimate the surface charge of droplets in the dispersion medium, and it is an indicator of droplet stability. Values exceeding +30 mV and –30 mV indicate good stability against coalescence (Kadu, Kushare, Thacker, & Gattani, 2011). Following the formation of particles, the charge increased drastically, indicating markedly higher stability. The zeta potential of zein–sodium alginate (–49.07 to –57.20 mV) and zein–pectin (–27.80 to –31.57 mV) changed to a higher negative charge (Table 1). The zein solution had a positive charge (+25.93 mV), whereas the anionic polysaccharides (sodium alginate and pectin) had a negative charge at pH 4.0. The anionic polysaccharide molecules were absorbed by the cationic zein surface via electrostatic interaction (Hu & McClements, 2015). For zein–chitosan, the interaction between zein and chitosan was achieved by rapidly mixing the antisolvent into the original solvent, resulting in the formation of small droplets. When the concentration of ethanol decreased below the solubilization limit, zein precipitated and formed positively charged particles with chitosan. The self-assembly of zein and chitosan led to partial complexation. The interaction between zein and chitosan via the antisolvent precipitation introduced the formation of hydrophobic moieties (Wang et al., 2016).

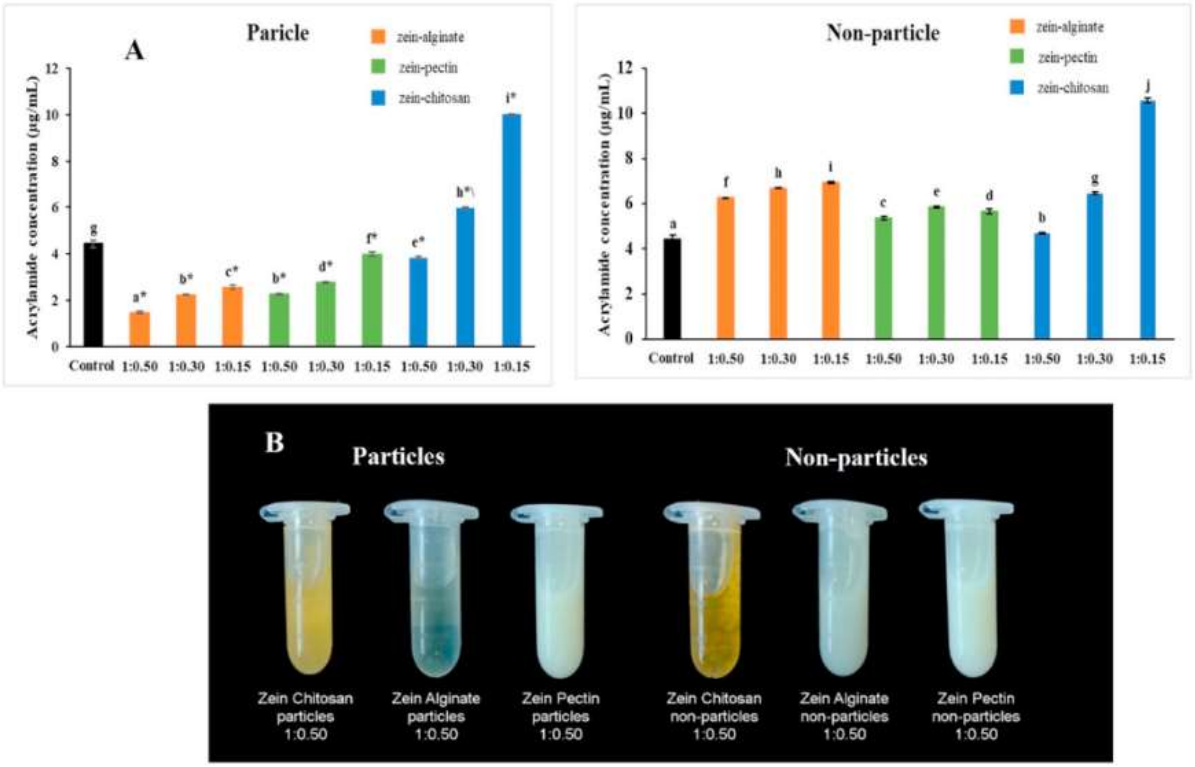


Fig. 3. (A) Acrylamide formation after using protein–polysaccharide complexes as inhibitors in a heating block. (B) Effect of zein–polysaccharide complex particles and non-complex particles (1:0.50) on browning formation at 170 ◦C for 30 min in a heating block. Data are presented as the mean ±standard deviation (n =3). $^{a–j}$ different letters indicating statistically significant difference ($p$ <0.05). * mean difference ($t$-test) between complex particles and non-complex particles.

The average size of zein–polysaccharide complex particles was larger than that of zein or polysaccharides alone, indicating the formation of complex particles. Higher polysaccharide ratios were associated with larger average particles size and higher stability (Table 1). It has been reported that the complex particle abolishes its compact structure because the polysaccharide concentration is not adequate for stabilizing a high protein concentration in the core or low surface coverage, which induces bridging flocculation (Dickinson, 2017). Thus, an appropriate ratio of zein and polysaccharide

for the complete coverage of zein particles could result in the formation of a perfect zein–polysaccharide core-shell structure. The highest particle stability was observed for zein–chitosan (48.5–61.3 mV), followed by zein–alginate (–49.07 to –57.20 mV) and zein–pectin (–27.80 to –31.6 mV); of note, the stability showed a negative correlation with the average particle size. The smallest average particle size was recorded for zein–pectin (401.7–592.4 nm), followed by zein–alginate (649.7–729.7 nm) and zein–chitosan (1181.3–2251.3 nm) (Table 1).

The sample with higher PDI would have a broader MW distribution. Generally, a PDI >0.4 indicated a broad distribution, whereas a PDI <0.1 denoted monodispersity of particle sizes (Mudalige et al., 2019). After the formation of the complex particle, the PDI was drastically reduced. The PDI of the complex particles was the smallest (0.13), indicating more uniformity and monodispersity compared with zein (0.32), chitosan (0.47), alginate (0.31), pectin (0.22), and non-complex particles (0.21) (Table 1). Higher zeta potentials and lower PDI were indicative of increased formation and higher stability of protein–polysaccharide complex particles.

To confirm the formation of complex particles, the particles were observed using confocal laser scanning microscopy at different wavelengths. The images showed particles of zein, colored green (Fig. 2A); chitosan, sodium alginate, and pectin, colored red (Figure 2B1, 2B2, 2B3), and zein–polysaccharide complex particles of mixed colors, red and green (Fig. 2C). All particles were noted at the same position. Orange colored complex particles were green zein particles mixed with red chitosan/polysaccharide particles. Imaging showed that zein–chitosan particles were much bigger (1181–2251 nm) than zein–alginate (650–730 nm), and zein–pectin particles (402–592 nm).

3.2.2. Inhibition of acrylamide by zein–polysaccharide complex particles

The zein–polysaccharide complex particles successfully reduced the concentration of acrylamide in a heating block model (170 ◦C, 30 min). The most effective inhibitor of acrylamide was zein–alginate (1:0.50) (1.45 ±0.17 μg/mL), followed by zein–pectin (1:0.50) (2.25 ±0.04 μg/ mL), versus control (4.43 ±0.17 μg/mL). However, the use of zein–-chitosan complex particles promoted acrylamide formation, particularly at lower concentrations of chitosan (Fig. 3A). Chitosan could not inhibit acrylamide formation to the same extent as alginate and pectin owing to its lower emulsion stability. The zein–polysaccharide ratio was essential for complexation because the lack of a sufficient amount of polysaccharide available to stabilize high protein concentration in the core caused the complex particles to deteriorate (Dickinson, 2017). This finding was consistent with that of our previous study, which demonstrated that the optimum amount of sodium alginate (0.3%) and pectin (0.2%) could reduce acrylamide formation in both conventional and microwave heating (Champrasert et al., 2021). The $Na^+$ in sodium alginate could inhibit acrylamide formation because the cations pre-vented the formation of asparagine and related intermediates (Gokmen & Senyuva, 2007; Lindsay & Jang, 2005). Pectin could inhibit acryl-amide by lowering the pH. Aside from the ion and pH effects, the presence of long chain polysaccharides could result in lower acrylamide formation by preventing the interaction of intermediates and slowing the motion of the molecules in the system (Passos et al., 2018). The free amino group of zein could compete with asparagine to bind with the carbonyl group of glucose, resulting in reduced production of acryl-amide (Anese, Suman, & Nicoli, 2009; Claeys et al., 2005).

The effect of the formation of complex particles on acrylamide inhibition was also examined by comparison with the solution of zein and polysaccharides by skipping the antisolvent precipitation step, resulting in no particles being formed (non-complex particles). The zein–alginate and zein–pectin particles exhibited markedly higher efficiency in acrylamide mitigation (1.45 ±0.05 μg/mL and 2.25 ±0.04 μg/mL, respectively) than their non-complex counterparts (6.26 ±0.01 μg/mL and 5.35 ±0.07 μg/mL, respectively) (Fig. 3A). The zein–polysaccharide complex particles showed less formation of

brown color than the non-complex particles (Fig. 3B). Different polysaccharides have been reported to reduce acrylamide formation in chemical (heating block and microwave heating) and food models, (Champrasert et al., 2021), but zein-polysaccharide complex particles of the same polysaccharides have been found to have stronger acrylamide inhibitory effects. A schematic image of the potential mechanism by which these particles inhibit acrylamide formation is presented in Fig. 4.

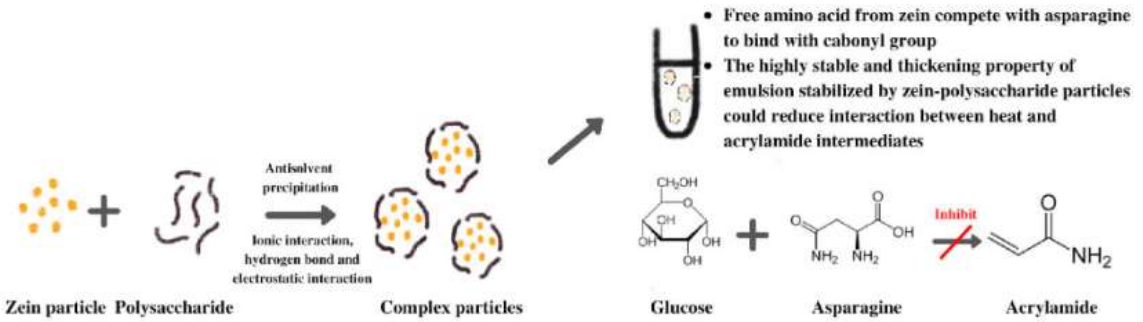


Fig. 4. Schematic image of the potential mechanism by which zein-polysaccharide complex particles inhibit acrylamide formation.

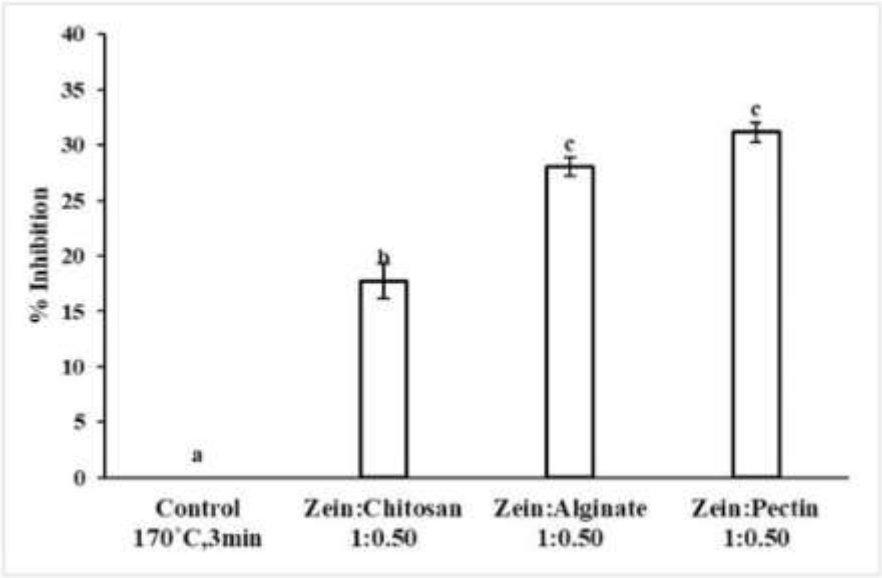


Fig. 5. Inhibition of acrylamide (%) in potato strips soaked in various zein–polysaccharides complex particles (1:0.5) for 30 min and deep fried at 170 ◦C for 3 min. $^{a–c}$ different letters indicating statistically significant difference ($p$ <0.05).

### 3.3. Acrylamide inhibition in a food model

Fried potato chips were used as the food model because of their wide consumption and high acrylamide content (0.25–2.67 mg/kg) (Capuano & Fogliano, 2011). According to the World Health Organization (2005), the daily dietary intake of acrylamide should be in the range of 0.3–2.0 mg/kg body weight. The inhibition of acrylamide in deep-fried potato strips dipped in zein–pectin (1:0.5), zein–alginate (1:0.5), and zein–-chitosan (1:0.5) compared with control was 31.16% ±0.05%, 28.05% ±0.09, and 17.75% ±2.24, respectively (Fig. 5). These results were similar to those obtained from the heating block model. The brown color of fried potato strips appeared to follow the formation of acrylamide. Maillard reactions involving asparagine produced acrylamide, and its concentration increased after cooking plant-derived foods (Mottram et al., 2002). The immersion duration was crucial in acrylamide mitigation because it causes the food surface tension to decrease and leads to the formation of a coating on the food surface (Zeng et al., 2010). The thermal gelation network of the

coating could reduce heat penetration from the oil into the food during frying, thus reducing any interactions that may occur between heat and acrylamide intermediates (Mousa, 2018; Suyatma, Ulfah, Prangdimurti, & Ishikawa, 2015; Zeng et al., 2010). The emulsions stabilized by protein-coated particles formed paste-like or gel-like networks (Hoffmann & Reger, 2014), which were stable and could thicken the system, thus facilitating the formation of a coating on the food surface and reducing the direct interaction between heat and intermediates. However, the effect of the different interfacial rheologies of these particles with the food surface on acrylamide inhibition requires further investigation.

## 4. Conclusion

The results of this study showed that the zein and polysaccharide complex could reduce acrylamide at high temperature in chemical and food models. The mixture of zein and polysaccharide (non-complex particles) was less effective in terms of acrylamide mitigation, emphasizing the unique properties of complex particles. Protein–polysaccharide complex particles are often investigated and applied to the field of encapsulation and emulsification. This study provided a new application of protein–polysaccharide complex particles for acrylamide mitigation.

## CRediT authorship contribution statement

Ornicha Champrasert: Methodology, Software, Data curation, Writing – original draft, Visualization. Caroline Orfila: Supervision, Data curation, Validation, Writing – review & editing. Prisana Suwannaporn: Conceptualization, Supervision, Data curation, Validation, Writing – review & editing.

## Declaration of competing interest

None.


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