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Inhibitory effect of polysaccharides on acrylamide formation in chemical and food model systems

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Abstract

The inhibitory effect of three polysaccharides (alginate, pectin and chitosan) on acrylamide formation was investigated in a chemical and a fried potato chip food system, under two heating regimes (heating block and microwave). In the chemical system, acrylamide formation followed a second order reaction kinetic behaviour. Activation energies (Ea) were 17.85 and 110.78 kJ/mol for conventional and microwave heating respectively. Acrylamide content was highest at 180°C after 60 min conventional heating (27.88 ng/ml) and 3.5 fold higher after microwave heating for 60 s (800W, 98.02 ng/ml). Alginate (0.3% w/v) and pectin (0.2% w/v) solutions efficiently inhibited acrylamide formation by 65% and 56% respectively under conventional heating, and 36% and 30% respectively under microwave heating. Coating potatoes with alginate, pectin and chitosan (1% w/v) prior to frying dramatically inhibited acrylamide formation by 54%, 51% and 41% respectively. However only alginate and pectin slightly reduced acrylamide by 5% in the microwave.

Keywords: Acrylamide; microwave; polysaccharides; pectin; alginate; chitosan; Maillard; potato

Chemical compounds:

Acrylamide (PubChem CID: 6579); Pectin (PubChem CID: 441476); Alginic acid (PubChem CID: 6850754); Chitosan (PubChem CID: 71853); Asparagine (PubChem CID: 6267).

1. Introduction

Acrylamide is classified as a group 2A carcinogen (probably carcinogenic to humans) by the International Agency for Research on Cancer (IARC, 1994). Acrylamide is not naturally present in foods, but can be formed during thermal food processing. Acrylamide is detected in heat processed starchy foods such as potato chips and fries, bakery products and roasted coffee (Krishnakumar and Visvanathan, 2014). The major pathway for acrylamide formation is the Maillard reaction between the α-amino group of free asparagine and carbonyl group of a reducing sugar (Mottram et al., 2002). A minor route is from the degradation of acrolein (Gertz and Klostermann, 2002). Mass spectral studies have shown that the three carbon atoms and the nitrogen atom of acrylamide are all derived from asparagine (Zyzak et al., 2003). Acrylamide formation is affected by many factors, such as the concentration and ratio of precursors (i.e. asparagine and reducing sugar), pH, water content, water activity, and physical process parameters such as heating time and temperature (Anese et al., 2009). The interaction between heating temperature and heating time was shown to be an important factor for acrylamide formation (Taubert et al., 2004). Most studies use heated surfaces to heat the material, which is mostly heat transfer by conduction from the hot surface to the heating vessel and subsequent convection within the material, particularly for liquids. Microwave heating is widely used in domestic and commercial food heating operations. Contradictory and non-conclusive results have been reported on the impact of microwave heating on acrylamide formation (Michalak et al., 2020). Recent studies indicated that microwave heating facilitates the Maillard reaction in a similar way to conventional heating such as boiling or frying (Maskan, 2001, Oliveira and Franca, 2002), and have shown acrylamide formation (Yuan et al., 2007, Zhang et al., 2008b). Other studies reported that acrylamide is not typically found in boiled or microwaved foods (Capuano and Fogliano, 2011, Krishnakumar and Visvanathan, 2014). The

contradictory results on acrylamide formation during microwave heating led to the conclusion that it may be difficult to control temperature during the experiment.

Although there is no set maximum limit for acrylamide content in foods, it is generally accepted that acrylamide levels in foods should be minimized as much as possible (Dybing et al., 2005). In order to reduce acrylamide formation during cooking, inhibitors may be used. Hydrocolloids such as pectin, alginic acid and xanthan gum were reported to significantly inhibit acrylamide formation during cooking (Zeng et al., 2010). Coating with hydrocolloid could reduce oil uptake and modify the heating properties of the food system during frying, with no apparent negative effects on sensory attributes (Kurek et al., 2017, Zeng et al., 2010).

The aim of this study is to investigate the inhibitory effect of three polysaccharides (citrus pectin, sodium alginate and chitosan) tested at various concentrations, on acrylamide formation in chemical and food model systems. In both systems, a comparative effect of different processing methods (conventional heating in a heating block and microwave heating) on the formation of acrylamide were examined under controlled temperature and time conditions. In contrast to previous research, we used a Microwave Accelerated Reaction System to carefully monitor temperature changes in model system in the microwave.

The hypothesis tested was: polysaccharides inhibit acrylamide formation in both chemical and food models under heating block and microwave heating.

2. Materials and methods

2.1 Acrylamide formation in chemical model system

a) Conventional heating

The reaction under conventional heating was carried out following the method described by Sansano et al. (2017) with minor modifications. In this study heating block was used to generate

heat instead of oil bath due to constant temperature. The mixture between asparagine (Fisher, AC371601000) and glucose (Sigma-Aldrich, G8644) was heated at equimolar amounts; 5µM glucose and 5µM asparagine (1.5 mL of each) were mixed in a Pyrex tube fitted with a screw cap. Samples were heated at 150, 160, 170 and 180°C in heating block (VWR analog Heatblock, Hampton, NH, USA) for 10, 20, 30, 40, 50 and 60 min. Heating block was pre-heated for 2 hours. The temperature fluctuation was within 2°C. The tubes were occasionally swirled manually. The heating block temperature was measured using an infrared thermometer. It was assumed that temperature in the heating block was identical to the temperature inside the tube by neglecting the conductive resistance to heat transfer. At the end of the heating time, the samples were cooled immediately in an ice bath to stop reaction for 5 min.

b) Microwave heating

The microwave heating was performed via a Microwave Accelerated Reaction System (Model MARS 6®, Matthews, NC, USA). In the microwave chamber, there were 10 microwave sample vessels in a rotating carousel, which allowed 10 simultaneous sample reactions under identical reaction conditions. The reaction temperature, time, and the control limits were modulated via a digital intelligent control panel. The reaction mixtures were treated at the constant microwave power of 800W with even wave administration (no hotspots). All reactions contained the same reactant concentrations as described for the conventional heating reactions and microwaved for 30, 60, 90 and 120 sec. The mixtures were then cooled immediately in an ice bath.

2.2 Acrylamide formation in food model system

a) Fried potato chips

A batch of potatoes (variety Maris Piper) was purchased from local market. Potatoes were washed, peeled and cut into the similar size strips (10 x 10 x 50 mm) prior to frying in a pre-heated deep fat fryer (Cookworks™) in sunflower oil at 170 °C for 3 min. Samples were cooled

and drained of excess oil on a metal sieve. Fried potato chips were ground using mini food processor.

Acrylamide extraction from food model was adapted from Gökmen and Şenyuva (2007). Ground food sample (1 g) was weighted into a 15 ml centrifuge tube. Carrez I and II (500 µL of each) and 9 ml of 1.2% (v/v) of acetic acid were added. Sample was mixed vigorously for 2 min. After mixing, 1ml of sample was centrifuged at 10,000 rpm for 10 min at 0°C (Centrifuge 5424R Eppendorf). Supernatant was collected and diluted with Milli-Q water (1:10) before HPLC analysis.

b) Microwaved potato chips

Potatoes were washed, peeled and cut into the similar size strips (10 x 10 x 50 mm) prior to heating in commercial microwave. Our preliminary experiment showed that at 3 min potato chips did not form any brown color, and 5 min would burn the potato chips. Therefore, microwave process was performed at 800 W for 4 min. All samples were cooled before grinding in mini food processor and extracted as previously described.

2.3 Kinetic calculation

The order of reaction and the reaction rate constant (*k*) for the formation of acrylamide were calculated from linear regression of the concentration and 1/concentration versus reaction time for zero, first and second order reaction kinetics, respectively. When linear graph was obtained, the natural logarithm of rate constant (*k*) was plotted versus 1/T (K unit) to calculate activation energy (Ea) using Arrhenius equation ($\ln k = \ln k_0 - E_a/RT$).

2.4 UV-Vis spectrophotometer measurement

A UV–VIS spectrophotometer (SPECORD 210 PLUS, Analytik, Jena, Germany) was used to monitor the reaction according to Zhou et al. (2016). Samples were diluted in water (1:50) and the absorbance was measured at 294 and 420 nm to determine the intermediate and final products of Maillard reaction, respectively.

2.5 Acrylamide determination by HPLC

Acrylamide was analyzed in triplicate following the method by Galani et al. (2017) with slight modifications. A HPLC system (Agilent 1200 series, Santa Clara, CA, USA) equipped with an auto sampler and a diode array detector was used. Two microliters of sample were injected by auto sampler and separated using a Zorbax 300 Extend C18 analytical column (2.1 mm x 100 mm, 3.5 μm) (Santa Clara, California, USA). Formic acid (Sigma-Aldrich, F0507) (0.1% in water) was used as mobile phase with flow rate of 0.1 mL/min. The running time was 5 min and retention time of acrylamide was at 3.4 min. Acrylamide solution (Sigma-Aldrich, A9099) (1 mg/mL) was prepared, then a series of acrylamide concentrations (1 to 100 μg/mL) were prepared from the stock solution. The position of the acrylamide peak in reaction mixtures was confirmed by spiking. All test samples were diluted in Milli-Q water to be within the standard range and filtered with Nylon filter (0.45 μm) (Sigma-Aldrich Co., St. Louis, MO, USA) and transferred to a vial for chromatography analysis.

2.6 Preparation of polysaccharide solutions

In chemical system, sodium alginate (Sigma-Aldrich, W201502) was dispersed in Milli Q water (1g in 100 ml) and heated at 70°C with stirring for 30 min, then cooled down at ambient temperature. Dilutions were made to 0.1% to 0.3% (v/v) (pH=4). Pectin from citrus peel (Sigma-Aldrich, P9135) was dispersed in Milli Q water (1g in 100 ml) and stirred overnight at room temperature. Pectin solutions were diluted to 0.1% to 0.3% (v/v) (pH=4). 1g of chitosan (Sigma-Aldrich, 448869) which had molecular weight at 50-190 kDa and 75-85% deacetylated degree was solubilized in acetic acid (1% v/v), 100 ml and stirred overnight until completely dissolve. Dilutions were made to 0.1% to 0.3% (v/v) with 1% acetic acid and adjusted pH to 4.0 by 0.1M and 1M NaOH.

In food system, 1% dilution of sodium alginate, pectin and chitosan were made using same method as describe above. Potatoes (50 g per portion) were soaked in inhibitor solution (50 ml) for 30 min and drained for 2 min before frying.

2.7 Statistical analysis

The data was subjected to analysis of variance (ANOVA) and least significance difference test to determine difference between means. Duncan's multiple range tests was used to compare means at a significance level of 0.05 using the SPSS software package version 24 (IBM Institute., New York, USA).

3. Result and discussion

3.1 Inhibitory effect of polysaccharides on acrylamide formation in a chemical model system

3.1.1 Acrylamide formation in chemical model system

Conventional heating in a heating block resulted in acrylamide formation under all temperature conditions, with the highest levels recorded at 180 °C after 60 min of heating (27.88 μg/mL) (

The formation of Maillard reaction intermediates was monitored with UV–Vis spectrometry at 294 nm and 420 nm (Figure 1). Absorbance values at 294 nm increased steadily with time at 150, 160 and 170°C, with a much steeper increase in absorbance values at 180°C after 40 min. These values correlate with acrylamide content in those conditions. Much smaller changes in absorbance values at 420 nm were observed, suggesting that 294 nm is a better wavelength to monitor the reaction. Microwave heating showed similar patterns of absorbance change.

1). There was a marked increase in the rate of formation after 40 min of heating at 180°C. Gökmen and Şenyuva (2007) reported that high levels of acrylamide were generated at elevated heating temperature (180°C) and heating time (10 min), and decreased slightly after 10min until 60min, likely due to degradation of acrylamide into other compounds. This decrease was not observed in this study.

When the same reaction mixtures were subjected to microwave heating at a power of 800W, the results also showed an increase in acrylamide with increased temperature and time (Figure 1). There was a similar pattern of acrylamide formation between conventional and microwave heating, however it must be noted that the timescale in the microwave experiment is in seconds, while the timescale in the conventional heating system is in minutes. It is important to note that the microwave reactor is designed to prevent hotspots, and therefore we are confident that acrylamide formation was uniform in the solution.

Microwaves cause fast temperature rises in the solvent due to their capacity to generate heat energy inside the system, without requiring any medium as vehicle for heat transfer. A low thermal conductivity product may quickly reach high temperatures (Campañone and Zaritzky, 2005). Moreover, microwaves causes molecular friction in alternating electrical and magnetic fields, resulting in dipolar rotation of polar molecule (such as water) and rapid heat generation (Campañone and Zaritzky, 2005, Mudgett, 1989, Orzáez Villanueva et al., 2000).

The formation of Maillard reaction intermediates was monitored with UV–Vis spectrometry at 294 nm and 420 nm (Figure 1). Absorbance values at 294 nm increased steadily with time at 150, 160 and 170°C, with a much steeper increase in absorbance values at 180°C after 40 min. These values correlate with acrylamide content in those conditions. Much smaller changes in absorbance values at 420 nm were observed, suggesting that 294 nm is a better wavelength to monitor the reaction. Microwave heating showed similar patterns of absorbance change.

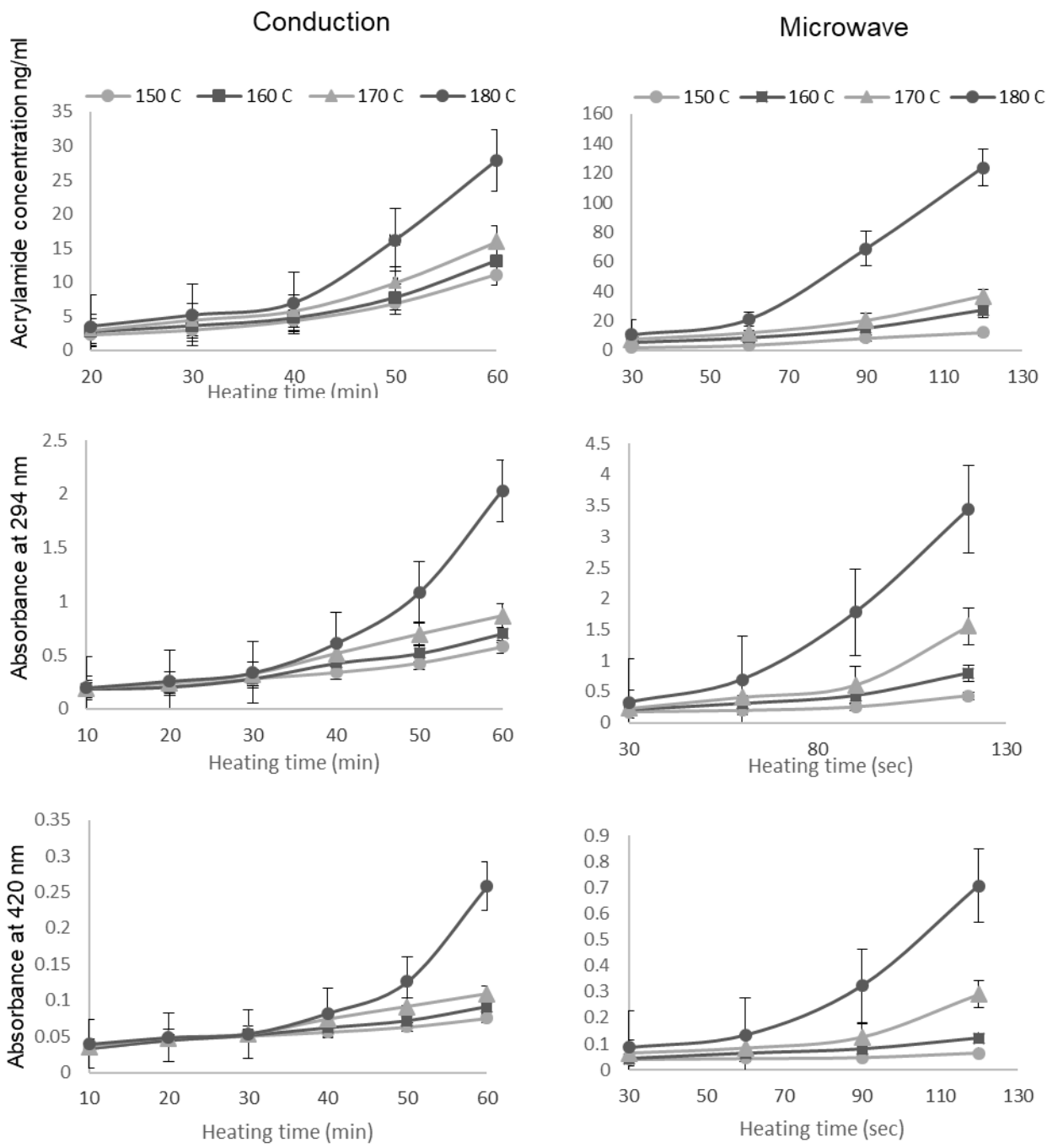

Figure 1. Acrylamide formation in the chemical model system during heating mainly by conduction in a heating block (conventional) and microwave at various temperatures. The absorbance of the solutions was measured at 294 and 420 nm.

3.1.2 Kinetic parameters

Acrylamide concentrations formed at different time-temperature combinations were used to calculate the kinetic parameters. The concentration of acrylamide formed at various heating

213 temperature was linear regressed against reaction time, and indicated that the reaction followed
214 a second-order reaction. This means that the reaction rate depends on the square of the
215 concentration of one or more reactants. This is consistent with previous observations by BeMiller
216 (2019), but contrasts with the results from Gokmen and Senyuva (2006). They used a fructose-
217 asparagine mixture to study acrylamide formation at 120-200°C. Their results show that the
218 reaction followed zero order and first order with respect to asparagine and fructose,
219 respectively. As the concentration of asparagine increased, the rate of reaction remained
220 constant.
221 Activation energy (Ea) was calculated using the Arrhenius equation from rate constants at each
222 temperature (

223 The formation of Maillard reaction intermediates was monitored with UV–Vis spectrometry at
224 294 nm and 420 nm (Figure 1). Absorbance values at 294 nm increased steadily with time at 150,
225 160 and 170°C, with a much steeper increase in absorbance values at 180°C after 40 min. These
226 values correlate with acrylamide content in those conditions. Much smaller changes in
227 absorbance values at 420 nm were observed, suggesting that 294 nm is a better wavelength to
228 monitor the reaction. Microwave heating showed similar patterns of absorbance change.
229). The apparent activation energy (Ea) was 17.85 (r^2 = 0.884) and 110.78 (r^2 = 0.829) kJ/mol for
230 conventional and microwave, respectively. This indicates that a higher Ea is needed in the
231 microwave heating conditions, but once activation energy is reached, the reaction progresses
232 rapidly.
233 Table 1. Kinetic parameters of acrylamide formation in the chemical model under different
234 heating conditions.

T °C	Condution heating		Microwave heating	
	k (s^{-1})	R^2	k (s^{-1})	R^2

150	0.0302	0.9882	0.0204	0.92822
160	0.0385	0.9740	0.0180	0.9966
170	0.0421	0.9881	0.0176	0.9961
180	0.0529	0.9688	0.0151	0.9512

235

236 3.1.3. **Inhibitory effect of polysaccharides on acrylamide formation**

237 The inhibitory effect of sodium alginate, pectin and chitosan on acrylamide formation were
238 investigated. Figure 2 shows that sodium alginate and pectin at 0.3% and 0.2% w/v reduced
239 acrylamide formation significantly in both conventional and microwave heating conditions
240 compared to control. At a concentration of 0.3% w/v, alginate and pectin reduced acrylamide
241 formation by 64.9% and 55.9% respectively in conventional, and 35.9% and 30% respectively in
242 microwave. Zeng et al. (2010) showed similar results with inhibition >50% for pectin and alginic
243 acid in conventional heating conditions, but with much higher solution concentration (2% w/w).
244 However, very low concentration of alginate and pectin (0.1% w/v) did not show as good
245 inhibition effect as higher concentration (0.2 - 0.3% w/v). The mechanisms by which sodium
246 alginate inhibits acrylamide formation have been proposed in previous studies. Gökmen and
247 Şenyuva (2007) found that Na^+ almost halved the acrylamide formation, because the cations
248 prevented the formation of key intermediates. Lindsay and Jang (2005) also suggested that ionic
249 associations involving the ions and charged groups on asparagine and related intermediates
250 were likely to be involved. Pectin effectively inhibited acrylamide by lowing the pH without
251 contributing to the reducing sugars content, and higher concentration of pectin (up to 5%) was
252 found to be more efficient in inhibiting acrylamide formation (Passos et al., 2018). Beside the
253 ionic and pH effects, the presence of long polysaccharide chains are likely to prevent substrates
254 coming together and slow down the motion of molecules in the chemical model system.

Chitosan did not inhibit acrylamide formation to the same extent as alginate and pectin. Only the highest concentration (0.3%) used slightly reduced (9.46%) acrylamide formation (Figure 2). Sung et al. (2018) found 1% chitosan reduced acrylamide formation by 46.8% and proposed that the amino groups of chitosan could compete with asparagine and react with the carbonyl groups of glucose in order to inhibit the formation of Maillard reaction products and acrylamide. Sansano et al. (2017) found that adding 0.5% of chitosan led to an inhibition of acrylamide formation by 52%, while 1% of chitosan could inhibit for 75% at 180 °C. On the other hand, Figure 2 shows that low concentration (0.2 and 0.1% w/v) of chitosan promoted acrylamide formation in conventional heating, with 0.1 % chitosan increasing acrylamide by almost five-fold compare to control. Samples containing low concentration of chitosan (0.2 and 0.1% w/v) carbonized in the microwave, hence acrylamide content could not be determined, but suggesting the reaction was taking place at a very high speed. Therefore, the concentration of chitosan is a critical factor to determine whether it inhibits or promotes acrylamide formation. Previous studies also suggested that different properties of chitosan would significantly influence its potential to inhibit acrylamide formation, including molecular weight (Mw) (Chang et al., 2016) and deacetylation degree (DD) (TSAI et al., 2002, Sansano et al., 2017). The chitosan used in this study had relatively low Mw (50-190 kDa) and high DD (75-85%), both of these properties have been shown to be associated with lower acrylamide formation (Sansano et al., 2017, Chang et al., 2016). However, our study also shows chitosan concentration is critical to the inhibition effect.

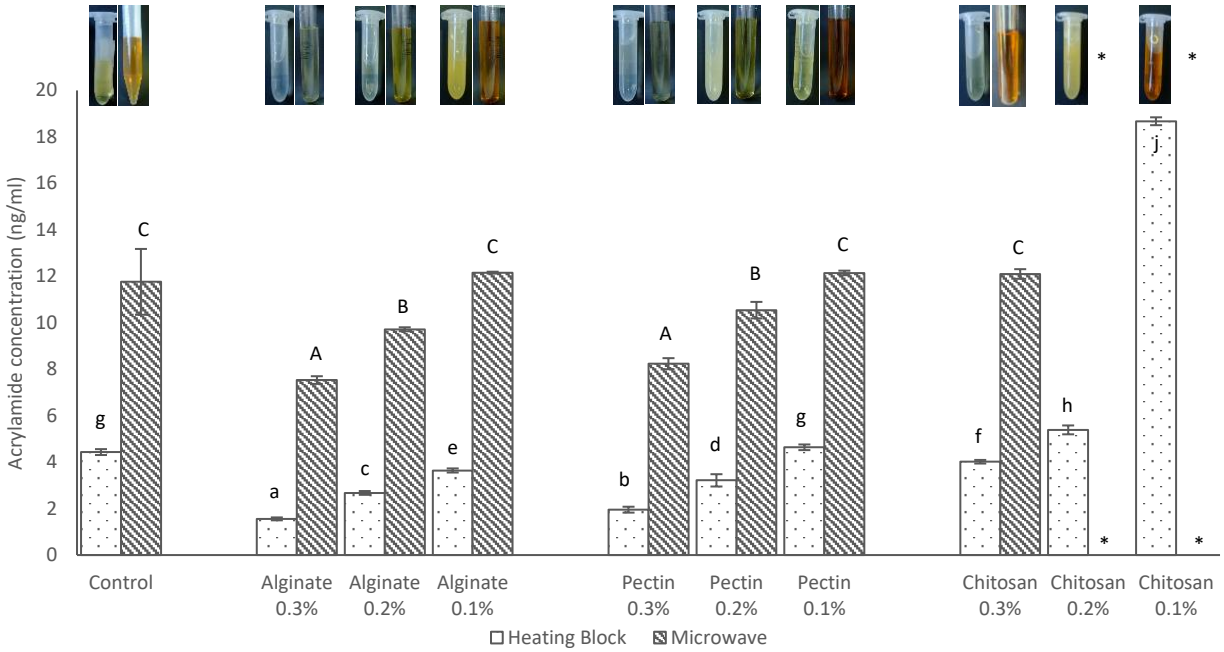

Figure 2 Acrylamide formation in conventional heating and microwave in the chemical model in the presence of various inhibitors. The conventional heating was carried out at 170 °C for 30 min. The microwave reaction was carried out at 800W microwave power for 60s. Data values are means ± SD (n = 3). Different lowercase letters indicate significant difference at the 5% level for conventional heating, and uppercase letters for microwave. *Samples containing low concentration of chitosan (0.2 and 0.1% w/v) carbonized in the microwave, hence acrylamide content could not be determined.

3.2 Inhibitory effect of polysaccharides on acrylamide formation in a food model (potato chips/fries)

To investigate the effect of polysaccharide inhibitors on acrylamide formation in a food model, potatoes were deep-fried or microwaved after dipping in polysaccharide solutions. Figure 3 shows the relative percentage of acrylamide in fried and microwave potatoes. Dipping in alginate, pectin and chitosan dramaticaly reduced acrylamide formation during frying by 53.5%, 51.2% and 40.9% respectively compared to control frying. Microwave cooking potato chips

showed about a third less acrylamide formation compared to frying, and this was associated with less browning at the surface. It must be noted that the crips were cooked in a domestic microwave, not the microwave reactor used in the food model. Domestic microwaves have less homogeneous wave distribution, and it is therefore possible that hotspots were created within the potato chip. However, our replicate analysis shows good reproducibility between samples, indicating homogenous acrylamide formation. However for microwave cooking, only alginate and pectin significantly reduced acrylamide formation and only by 5.2% for both polysaccharides. Previous studies on the effect of microwave cooking on acrylamide formation have been contradictory (Michalak et al., 2020). Some studies reported that microwave heating provides a favorable medium for the occurrence of acrylamide and promote the acrylamide formation (Michalak et al., 2017, Yuan et al., 2007, Zhang et al., 2008a), but other studies showed no acrylamide formation under microwave heating (Anese et al., 2013, Barutcu et al., 2009). Others suggest that microwave heating prior to frying may help to reduce acrylamide formation (Belgin Erdoğdu et al., 2007). The differences might be due to variations in microwave parameters such as power or heating time, as well as the chemical composition and water activity levels (Michalak et al., 2020).

Zeng et al. (2010) showed slightly different pattern for alginate and pectin in their food model compare to the current study. They found that 1% w/w alginic acid only lead to about 20% reduction of acrylamide, 1% pectin caused slightly elevated acrylamide contents and only efficiently inhibited acrylamide after 5h immersion (Zeng et al., 2010). They suggest that duration of immersion played a predominant role in determining the final effect of the treatment (Zeng et al., 2010). Sansano et al. (2016) found low concentration of chitosan (0.27% w/v) mitigated acrylamide formation by around 60% in batter system for frying, but did not inhibit when concentration increased to 0.54% w/v. Our results shows all three polysaccharides at 1% w/w

efficiently reduced acrylamide formation, with alginate giving the best result followed by pectin and chitosan.

Gaonkar (1991) explained that immersion of hydrocolloid solution would lower the food surface tension and thus facilitate the formation of a layer of coating on the surface of the food products. Mousa (2018) confirmed that a rigid thermal gelation network formed during frying and prevent interaction between acrylamide precursors. Zeng et al. (2010) and Suyatma et al. (2015) also explained that pectin coating reduces heat penetration from the oil to the food during frying and interact with acrylamide precursor. The mechanism of inhibition needs to be further studied to confirm the role of hydrocolloids in influencing the acrylamide formation in food model systems. The coatings did not seem to impact on colour formation or other aspects of appearance, compared to control. However, the effect of the coatings on other sensory properties (flavour, aroma) needs further investigation.

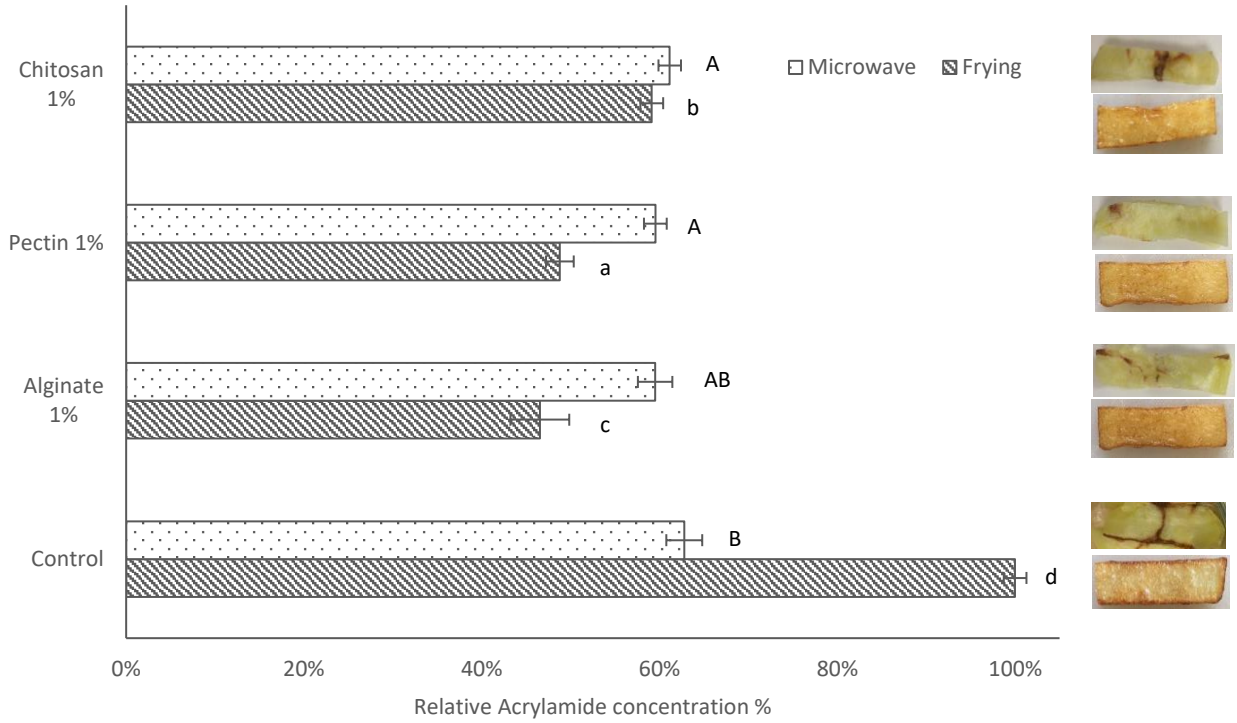

Figure 3. Acrylamide formation in the food model in the presence of various inhibitors relative to control (frying with no inhibitors). The microwave cooking was carried out at 800W power for 4 min. Frying was carried out at 170°C for 3min. Data values are means ± SD (n = 3). Different lowercase letters indicate significant difference at 95% confidence level for frying, uppercase letters for microwave compared to the control.

4. Conclusion

In this study, the inhibitory effect of polysaccharides on acrylamide formation was investigated in chemical and food model systems under two heating modes (heating block and microwave). Low concentration of polysaccharides (0.2 – 0.3% w/v) successfully inhibited acrylamide formation in the chemical model, although chitosan was less effective compared to sodium alginate and pectin. Low concentrations of chitosan (0.1%) increased acrylamide formation. In the food system, the three polysaccharides (1% w/v) reduced acrylamide by up to 53.5% during frying. However the inhibition was much lower when the chips were microwaved (only 5.2%). Alginate was shown to be the most effective inhibitor of acrylamide formation in both chemical and food model, followed by pectin, although the mechanism behind the inhibition needs to be further investigated. Dipping in polysaccharide solution prior to frying is a promising acrylamide mitigation strategy.

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The authors declare no conflicts of interest.

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