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Using particle size and fat content to control the release of Allyl isothiocyanate from ground mustard seeds for its application in antimicrobial packaging

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ABSTRACT

In this study an active antimicrobial packaging based on the controlled release of Allyl isothiocyanate (AITC) from mustard seed was designed. The effect of fat content and particle size of ground mustard seeds on formation and release of AITC was investigated and the underlying mechanisms were highlighted. A smaller size of mustard particles resulted in more sinigrin conversion to AITC and a higher release of AITC in the headspace. The fat content has an important role on AITC release, a decreased fat content decreased AITC levels in the particles and increased the amount of AITC in the headspace. Based on the results of the sinigrin hydrolysis, the AITC surface exchange rate and the AITC fat solubility, an overall picture of the factors influencing the AITC release from the particles is proposed, which describes formation of AITC and its partitioning between the compartments of the particles and the headspace.

1. Introduction

Consumer demand for fresh and safe food products is increasing (Tilman & Clark, 2014; Wier, O'Doherty Jensen, Andersen, & Millock, 2008). However, fresh food products create perfect circumstances for the growth of a variety of microorganisms that can spoil the food during storage (Buisman, Haijema, & Bloemhof-Ruwaard, 2017). The food spoilage often starts at the food surface and active packaging that releases volatile antimicrobials into the headspace can effectively inhibit the growth of spoilage organisms at the food surface (Jin, 2017). These spoilage bacteria cause the food products to be unacceptable for consumers from a sensory point of view and finally wasted. The control of spoilage bacteria by volatile antimicrobials is therefore commercially and sustainably interesting to retain the product quality for a longer time. The extended shelf life will help to reduce food waste which is a huge global problem in terms of sustainability and food security (Garnett, 2013).

Allyl isothiocyanate (AITC) has strong antimicrobial activity against a wide variety of spoilage and pathogenic microorganism at low concentration (Kurek et al., 2017). The presence of AITC in a vapour phase (indirect contact) is more effective than their direct addition to food product (Lin, Preston, & Wei, 2000). At 2.5 ppm in the headspace, AITC vapour completely inhibited the growth of filamentous fungi, such as *Aspergillus parasiticus* (Lopes et al., 2018). AITC vaporised from pure mustard essential oils (3–10 µl) fully inhibits the growth of nine foodborne bacteria strains, including *S. enterica, S. aureus, B. cereus* and *E. coli OI57:H7* (Clemente, Aznar, Silva, & Nerín, 2016) and ten mould strains, *A. flavus, B. fuckeliana, P. roqueforti,* etc, had been completely killed by 10 µl AITC (Clemente, Aznar, & Nerin, 2019). AITC (25–50 µl/g) coated on acidified k-carrageenan/chitosan also reduced the growth of lactic acid bacteria (LAB) by 1.6 $\log_{10}$ CFU/g on chicken slices (Olaimat & Holley, 2016). Another way of application has been studied by using a sachet containing AITC (0.215 ppb) which was sufficient to reduce *Aspergillus flavus* (Otoni, Soares, da Silva, Medeiros, & Baffa, 2014). However, the use of pure AITC is restricted in food packaging due to the extreme pungency and high volatility, which causes the AITC to quickly release into the food, interacting with food components and providing unpleasant off flavour (Dufour, Stahl, & Baysse, 2015).

Mustard seeds can be used as a natural source to slowly release AITC into the headspace (Dai & Lim, 2014). This seed contains high concentration of glucosinolates, which are the nitrogen and sulphur-containing metabolites acting as AITC precursors. Glucosinolates are present in plants of the Brassica family, like cabbage, broccoli, horseradish and wasabi (Dekker, Hennig, & Verkerk, 2009). The main glucosinolate compound found in mustard seeds is sinigrin, and AITC is enzymatically formed when sinigrin is hydrolysed in humid environment by the

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endogenous plant enzyme myrosinase. Myrosinase is located in separate cells and can come into contact with sinigrin upon cell damage, e.g. during milling (Hanschen, Kühn, Nickel, Rohn, & Dekker, 2018). Mustard seeds contain around 39–42% fat (Antova, Angelova-Romova, Petkova, Teneva, & Marcheva, 2017; Olgun et al., 2017) and the AITC released into the headspace from a fatty mustard seeds has been described to be lower than that from defatted seeds (Dai & Lim, 2014). The particle size of ground mustard seeds is expected to affect both the cell damage and the surface area that is available for AITC release. We therefore hypothesized that manipulation of the fat content and particles size is a way to optimise the release of AITC in the headspace of a mustard based active antimicrobial packaging.

The release rate of AITC in a food packaging headspace will have consequences for the microbial growth inhibition in packed food (Gao et al., 2018). When the concentration of released AITC drops below the minimum inhibitory concentration (MIC), the inhibitory action of microbial growth will stop; consequently the spoilage on the food surface starts and the shelf life is no longer extended (Appendini & Hotchkiss, 2002). In principle, shelf life can be extended by gradually releasing an antimicrobial compound and maintaining it at concentrations above the MIC value for sufficiently long time. In this study, the controlled release of AITC in a food packaging environment was monitored by measuring the concentration of AITC released from ground mustard seeds into the headspace. We investigated the effect of particle sizes and fat content of the mustard seeds on the AITC release kinetics in order to understand the mechanism of the release of AITC in a food packaging headspace.

2. Materials and methods

2.1. Materials

All chemicals used in this study were analytical grade and purchased from Sigma Aldrich or Merck. The mustard seeds (*Brassica juncea*) were purchased from natuurproduct.com, *Jacob Hooy Brown Mustard seeds*.

2.2. Sample preparation

The fresh mustard seeds were weighted and then freeze-dried for 5 days (Christ Alpha 1–4 LD plus). The freeze-dried seeds were placed in desiccators for 15 min and then stored in the flask at −20 ^{0}C till usage. Afterwards, the seeds were milled in liquid nitrogen by using a milling machine (Fritsch; Pulverisette 14) at 12.000 rpm at temperature as low as possible. All collected samples were <1 mm. The samples were collected and divided into four size ranges (50–100, 200–315, 400–500 and 630–800 μm) by using different sieves (Hosokawa Alpine Air Jet sieve 200 Ls). The seeds were defatted by the Soxhlet extraction method. Diethyl ether (boiling point of 35 °C) was used as a solvent to avoid inactivation of myrosinase above 40 °C (Okunade, Ghawi, Methven, & Niranjan, 2015). Different extraction times and number of cycles were used to reach different fat contents; 29.1% (full-fat), 17.1% (partly-defatted), 2.8% (nearly-defatted), and 0.0% (fully-defatted). To determine the fat content of the samples, the flasks were weighted to determine the amount of extracted oil, thereby calculating the corresponding fat content in the ground seeds.

2.3. Determination of sinigrin concentration

The method of determining the sinigrin content in mustard seeds with hot methanol as a solvent was modified from Oliviero, Verkerk, and Dekker (2012). Around 0.1 g of mustard seeds were added to a 15 ml grainer centrifuge tube, and at the same time methanol with concentration 70% and 100% were incubated at 75 °C in water bath. A 2.4 ml of the 100% preheated methanol was added to each sample, then followed by 0.2 ml of 3 mM glucotropaeolione as internal standard (IS). The tubes were incubated at 75 °C for 20 min, while being vortexed every 5-minutes. Afterwards, the tubes were centrifuged (Thermo scientific; Heraeus Multifuge X3 R centrifuge) at 2500 rpm for 10 min at 4 °C.

After centrifugation, the supernatant was taken into a new tube, while the precipitate was re-extracted twice with 2 ml of 70% preheated methanol and those supernatants were combined to the previous supernatant. Around 6 ml of supernatant in total was stored at −20 °C until desulphation. In the desulphation, diethylanimoethyl sephadex (DEAE) columns were prepared firstly by dispersing 10 gr DEAE into 80 ml 2 M acetic acid and then incubating them overnight. Glass wool was added into a 2 ml syringe with a height of 1 cm, and then followed with 1 ml of the DEAE Sephadex ion exchanger solution and 1 ml the swollen DEAE. The DEAE column was washed twice with 1 ml of saturated water, and then 2 ml of the desulphated supernatants was added. To determine the retention time of sinigrin and glucotrupaeolione, a solution of sinigrin (Sinigrin hydrate ≥ 99%) and glucotropaeolione (KVL Denmark, 95%) was made and then added into another DEAE column. The columns of sample and reference were washed twice with 1 ml of sodium acetate 0.02 M. Afterwards, the syringes were transferred into new glass tubes. 75 μl of sulphatase enzyme solution was finally added into the syringe, which was then covered by parafilm. Those tubes were incubated overnight at room temperature.

The desulphated sinigrin were eluted from the columns by adding 0.5 ml milliQ water three times through a 25 mm syringe filter (phenomenex®; PHENEX.™-RC 45 syringe filter) into amber HPLC (High-Performance Liquid Chromatography) vials. The eluates were analyzed by reversed-phase HPLC (Thermo Scientific; UHPLC + focused Dionex Ulti-Mate 3000). The HPLC column used was A Lichrocart 125–4 column (Merck) at a flow rate of 1 ml/min with an injection volume of 20 μl. 100% milliQ water and 100% acetonitrile were used with as eluent A and B, respectively. UV–vis detector was set at 229 nm. Finally, the total running time was 26 min with a gradient elution as follows: 100% A and 0% B for 1 min, then in 20 min to 0% A and 100% B, and in 5 min back to 100% A and 0% B. The result was then processed with Chromeleon 7. Sinigrin and IS were identified by comparing the retention time and the spectra with the reference, sinigrin and glucotropaeolin. Quantification of the both was done with the help of the IS and the relative response factor (RRF) of sinigrin (1.053).

2.4. Determination of Allyl-isothiocyanate (AITC)

AITC was determined by the method of Oliviero, Verkerk, Van Boekel, and Dekker (2014) with minor adjustments. Around 100 mg of samples was weighed into a 15 ml Greiner centrifuge, then followed by 5 ml of dichloromethane (DCM) and 150 μl of internal standard (100 mM butyl isothiocyanate in MeOH). The samples were then centrifuged (Thermo Scientific; Heraeus Multifuge X3 R Centrifuge) at 3000 rpm for 5 min. The supernatant was collected by a Pasteur Pipette and then filtered into Klimax tubes through SPE columns with a help of a vacuum manifold fitted with a pump. The SPE columns (Strata® C18-E (55 μm, 70A), 3 ml SPE Tubes (0.5 g)) were then washed with 1 ml of DCM. At the same time, the reagent was daily fresh prepared by adding 110 μl of 200 mM butanethiol and 14 μl of 20 mM triethylamine to 5 ml of DCM. 500 μl of the eluate and 25 μl of the reagent were transferred into the HPLC vials and then vortexed. Those HPLC vials were incubated for an hour at a heating block at 30 ^{0}C while being vortexed every 15 min. After incubation, the solvent was evaporated by using a Nitrogen (N_2) stream. The samples were finally re-dissolved in 750 μl of ACN and MilliQ 1:9. To quantify the concentration of AITC, calibration standards were then prepared using a 95% pure AITC standard solution dissolved in DCM. The range of used concentrations was 1 μM – 10,000 μM. The DCM in the calibration standards was also evaporated with the N_2 stream and redissolved in 750 μl ACN: MilliQ (1:9).

The samples were analyzed using reversed phase HPLC (Thermo Scientific; UHPLC + focused, Dionex Ultimate 3000) with 100% MilliQ

(+0.1% formic acid) and 100% acetonitrile (+0.1% formic acid) as respective eluents A and B. An RP-18 column (Xbridge shield RP18; 3.0× 100 mm, 3,5 µm Waters) was used at a flow rate of 0.4 ml/min and an injection volume of 10 µl. The UV–Vis detectors were set to 274 nm, 290 nm and 240 nm. The total running was also 26 min with a gradient elution as follows: 80% A and 20% B for 1 min, then in 20 min to 5% A and 95% B, and in 5 min back to 80% A and 20% B. The results were then processed using Chromeleon 7.2. The amount of AITC in the samples was finally quantified by the curve of the calibration standards.

2.5. Determination of headspace AITC

Headspace AITC determination was performed by using GC-FID (Thermo-Scientific, Focus GC) and autosampler (Thermo-Scientific, TriPlus Autosampler), combined with SPME (100 µm polydimethylsiloxane, red fibre, 23ga). 0.16 g of samples were added in Duran bottles of 10 ml and rehydrated with 0.132 ml of milliQ water (ratio; 1:0,825). The AITC release in the vial headspace was automatically measured in triple overtime by the GC analysis at room temperature (~20 °C). A Restek Rxi-5HT GC column (30 m, 0.25 mm internal diameter, 0.25 µm stationary film thickness) was used with an inlet temperature of 250 °C, a splitless mode for 1 min (flow 10 ml/min). The oven was set at 40 °C for the first minute, with a temperature ramp of 10 °C/min increasing till 280 °C. The total running time was set at 26 min with 1 ml/min Helium gas as a carrier. The detector was set at a constant temperature of 270 °C, with a flow of 350 ml air and 35 ml of H_2. Analysis of the AITC was performed with Xcalibur software, in which AITC is known to be detected as two separate peaks (Malabed & Noel, 2014; Marton & Lavric, 2013). The calibration was quantified using pure AITC (Allyl isothiocyanate, 97%) dissolved in Hexane, with the range of used concentrations 1 ppm–250 ppm. To calculate the concentration of AITC in the headspace the SPME values were converted by using an experimentally determined relation between the SPME and the direct injection of headspace samples and a calibration with known amounts of AITC in hexane using liquid injection.

2.6. Confocal microscopy

This microscopy method was adopted from Zahir, Fogliano, and Capuano (2018) with few adjustments. Dye Nile red (0.05 wt%) and fluorescent dyes calcofluor white (0.002 wt%) were prepared to stain the cell wall and oil body measurement in the ground seeds. The dye mixture (30 µl) was added to the homogenised ground particles (30 µl) placed in a glass slide. The prepared samples were visualized by a confocal scanning laser microscope (LSM) type 510 (Zeiss, Oberkochen, Germany) using 405 nm blue/violet diode laser for calcofluor white and Nile red with a EC Plan-Neofluar 20×/0.5 A lens. All pictures were analyzed with ZEN blue edition (Carl Zeiss Microscopy).

2.7. Statistical analysis

Results were expressed as the mean values ± standard deviations (SD). Significant differences among samples were analyzed with analysis of variance (one-way ANOVA with Tukey post hoc test), using the statistical software R studio. The significance level of the test statistic (p) was set at $p < 0.05$.

3. Result and discussion

3.1. Morphology of ground mustard seeds

The degree of cell damage upon grinding mustard seeds is shown in Fig. 1 for representative mustard particles of selected sizes and fat contents. The micrographs of mustard particles with 29% fat (Fig. 1a and b) show that most cells remained intact after milling and a strong adhesion between cells was present. The damaged cells can only be seen on the surface of the ground seeds. The damaged cells at the particle surface clearly demonstrates that for the bigger particles (Fig. 1a) more intact cells remain compared to the smaller particles (Fig. 1b), since the small particles have a larger surface to volume ratio. The defatted particles (Fig. 1c and d) show more ruptured cells, both internal and on the surface of the particles. Most of the cells seem to be damaged during the fat extraction (Wang, Liang, & Yuan, 2011), which disrupts cell membranes and result in permeabilization of the cell wall (Ando et al., 2016; Hanschen et al., 2018). Interestingly, a small amount of lipids was still observed in apparently intact cells in the core of particles.

Cell damage is connected to glucosinolate degradation: sinigrin, the substrate in the cell of mustard seeds, is located in the vacuoles of the cotyledon cells while myrosinase can be found in separate myrosin cells (Nakano, Yamada, Bednarek, Nishimura, & Hara-Nishimura, 2014; Stauber et al., 2012). Upon tissue damage myrosinase are released from the myrosin cells and can hydrolyse sinigrin into antimicrobial AITC (Nakano et al., 2014; Okunade et al., 2015).

3.2. The effect of particle sizes and fat content on sinigrin degradation

In Fig. 2 the effects of the enzymatic degradation of sinigrin in mustard seeds having different particles and fat concentrations are shown. Results showed that the size of the particle significantly influenced sinigrin degradation in the full fat particles (29.1% fat) (Fig. 2a). The large particles (400–500 and 630–800 µm) showed a much slower and lower sinigrin hydrolysis than the small particles (50–100 and 200–315 µm). Only around 30% of sinigrin in the large particles was degraded during 3 h, compared with the small particles in which around 45% of sinigrin was degraded in the same time. The main effect of the particles size in the full-fat particles is the remaining sinigrin after 48 h. Over 60% of sinigrin remained in the large particles, while only 30% of sinigrin remained in the small particles. The non-hydrolyzed sinigrin is likely due to the incomplete cell damage during milling as supported by the micrographs shown in Fig. 1a and b. The bigger particles have less cell damage and therefore more sinigrin is not accessible to the myrosinase action. A significant increase of glucosinolate transformation with decreasing the particle size was also reported by Sharma, Ingle, Singh, Sarkar, and Upadhyay (2012). The results are quite different in the defatted ground seeds (0% fat) as shown Fig. 2b: in this case, no significant difference in the percentage of converted sinigrin was observed in the large and small particles. After 3 h, over half of sinigrin in the defatted particles was already degraded for all sizes. The sinigrin concentration remained stable between 24 and 48 h, and only 15% of sinigrin was left after 48 h for all particle sizes. It can be concluded that particle sizes strongly influences the sinigrin degradation in full-fat samples due to different ratio between surface area and volume of the particles, while small impact was found in defatted seeds due to the high cell damage that occurred during defatting.

In Fig. 2c, the effect of fat content on sinigrin degradation was shown. The fat-containing particles (2.75, 17.11, and 29.1%) show lower sinigrin degradation compared to the defatted seeds (0%). In defatted seeds, around 90% of sinigrin was enzymatically hydrolysed by myrosinase. The fat content is the most important factor influencing sinigrin degradation. The fat has induced less cell damage and have protected the intracellular cells of the ground seed from milling process, which means only extracellular cell was damaged by milling process as depicted Fig. 1b. In Fig. 1c and d, more cell damage in fully defatted samples was caused by the solvent effect during fat extraction. Once the cell is damaged, myrosinase can easily diffuse into the cells to hydrolyse sinigrin, thereby forming AITC which is subsequently released in the headspace (Van Eylen, Indrawati, Hendrickx, & Van Loey, 2006).

3.3. The effect of particle sizes on AITC formation in the seeds and release into the headspace

Fig. 3 shows the effects of particle sizes of the defatted and full-fat

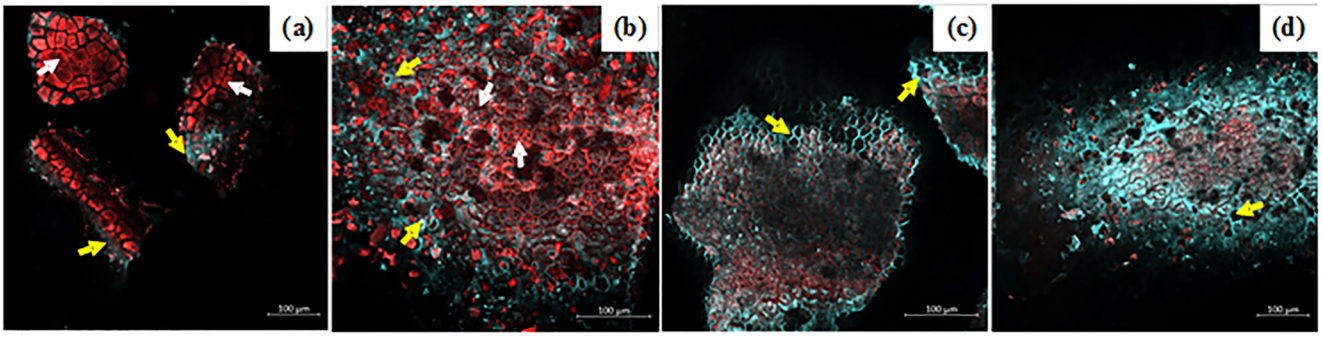

Fig. 1. Confocal laser scanning micrographs of mustard particles with 29% fat (a and b) and 0% fat (c and d). Cell wall (depicted in blue) and fat (depicted on red) were stained with calcofluor white and nile red respectively. Intact cells in all pictures are indicated by the white arrows, whereas cell damage was indicated by yellow arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

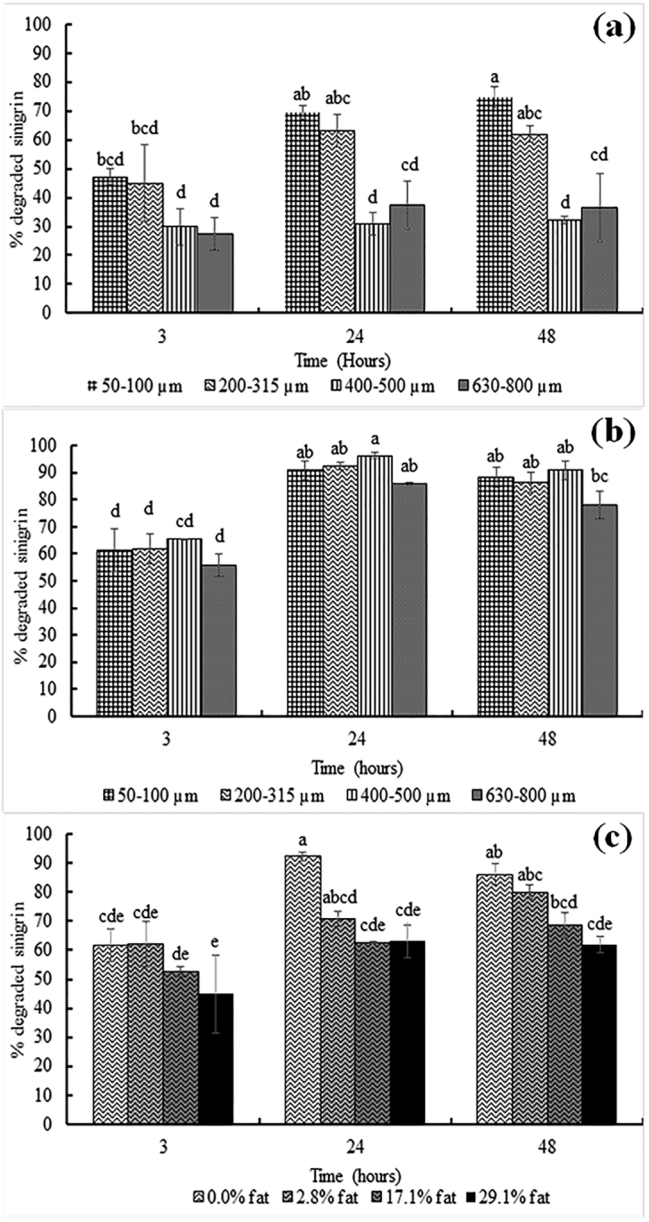

Fig. 2. Percentage of degraded sinigrin in (a) ground defatted (29.1% fat) and (b) full-fat mustard (0% fat) with different size ranges, and (c) ground mustard seeds (size 200–315 µm) with different fat contents. Different lower case letters indicate significant differences ($P < 0.05$).

ground seeds on AITC concentration in the particles and headspace. In Fig. 3a, the initial AITC concentration, before hydration, was in the range 30–55 µg/g in the full-fat particles, probably due to residual moisture that induces some myrosinase activity during milling (Dekker et al., 2009; Nakano et al., 2014). After 1 day, the concentration of AITC in the particles peaked at 70–93 µg/g and then decreased until day 9 where all particle sizes reached concentrations in the range of 4–7 µg/g. In the defatted particles (Fig. 3b) the AITC concentration was at a low level over time. The initial AITC concentration was below 10 µg/g. This might be an indication that in the defatted seeds, almost all of the AITC is directly released into the headspace after formation or it is lost by dissolving in the solvent during fat extraction. When the particles contain fat, the formed AITC is expected to be solubilized into the fat phase (Liu & Yang, 2010). It can be concluded that the particles size has no significant influence on the AITC concentration in the seeds.

For the AITC release to the headspace the particle size has a strong effect for both the full-fat (29.1% fat) and defatted particles (Fig. 3c and d). Smaller particle size resulted in higher AITC release in the headspace. Initially, no volatile AITC was detected in the headspace; however, after rehydration, the concentration of AITC in the headspace increases quickly within three hours. Preliminary experiment (data not presented), showed the concentration of AITC raised immediately after the rehydration and reached the highest levels already between 45 and 90 min. Fig. 3c shows that in three hours around 15 and 8 µg/l of AITC was present in the headspace from the small particles (50–100 and 200–315 µm, respectively); while AITC from the bigger particles (400–500 and 630–800 µm) in the headspace was much lower around 4 and 3 µg/l, respectively. These AITC headspace concentrations were lower than those obtained from the defatted seeds (0% fat). In Fig. 3d, in three hours the concentrations of AITC in headspace reached around 28–40 µg/l (for the particle sizes: 50–100, 200–315 and 630–800 µm). The smaller particles released higher headspace AITC, because of the higher surface-to-volume ratios of the seeds (Dai & Lim, 2014), which cause both fast diffusivity of moisture into the particles as well as of AITC out of the particles. This particle size effect was also reported by Dai and Lim (2014), who observed increased AITC release rates from ground mustard particles, compared to their unground counterparts. After a rapid peaking, the headspace concentration from all samples gradually dropped to 48 h. This gradual drop can be explained by AITC degradation in the headspace, due to its hydrolysis by presence of moisture in headspace at ~20 °C (Tsao, Yu, Friesen, Potter, & Chiba, 2000). In the headspace AITC degradation occurs due to an attack of nucleophiles, e.g. OH−, amino groups, and water, that can interact to functional N=C=S groups of AITC to generate other compounds, e.g. allyl amine and carbonyl sulphide (Dias et al., 2013; Tsao et al., 2000).

The defatted particles with a size 400–500 µm (Fig. 3d) resulted in much higher headspace AITC. The much higher increase in volatile AITC is difficult to explain, it might be caused by a specific optimal seed hydration due to the capillary forces being higher for this particular

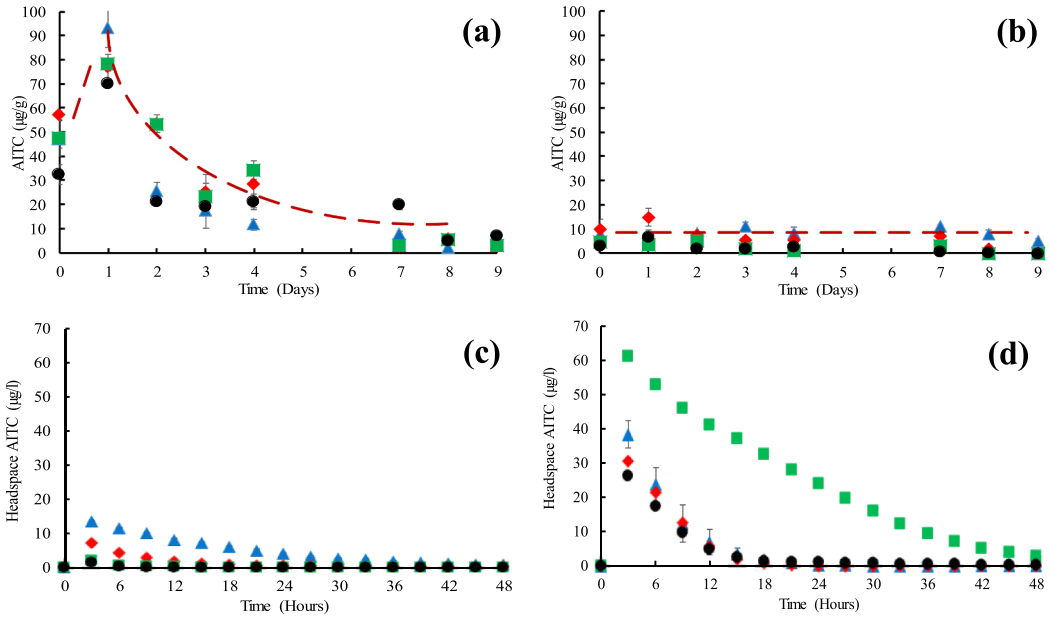

Fig. 3. The effect of particle sizes: (▲) 50–100 µm; (♦) 200–315 µm; (■) 400–500 µm; and (●) 630–800 µm on Allyl isothiocyanate (AITC) concentration in the (a) full-fat seeds (29.1% fat) and (b) defatted seeds (0% fat) for 9 days, and headspace AITC released from (c) full-fat seeds (29.1% fat) and (d) defatted seeds (0% fat) during 48 h.

size, leading to faster sinigrin degradation in the sample. As shown in the Fig. 2 in this sample the highest initial sinigrin degradation (almost 50%) at 3 h was observed. After 48 h, the percentage of degraded sinigrin was around 93%, which was higher than that of other samples. This figure suggests that an optimal release of AITC in the headspace can be obtained by regulating specific particle properties. The specific effects of particle structure and size on rehydration rate is also observed for dried powder of fruit and vegetables (Karam, Petit, Zimmer, Baudelaire Djantou, & Scher, 2016).

3.4. The effect of fat content on AITC formation in the seeds and release into the headspace

Fig. 4 shows the effects of different fat contents of the ground mustard seeds on AITC formation and release. A higher AITC concentration in the higher fat-containing particles was observed. This is likely caused by AITC migration to the fat phase in the seeds, which will improve its stability (Tsao et al., 2000). The higher remaining AITC in the fat phase (shown in Supplementary data). The calculation indicates that the majority of the formed AITC will be in the fat phase of the particles. It is clearly shown that the decrease of the fat content leads to the decrease of AITC concentration in the particles. Fig. 4a also clearly shows the stability of AITC in fat phase. The amount of headspace AITC can only be maintained for 33 h, compared to the particles with the higher fat content that can be maintained in longer time around 3 mg/g for 9 days. This prolonged effect was caused AITC is naturally oil soluble, which means that its chemical hydrophobicity is greater than its hydrophilicity (Giroux, Perreault, & Britten, 2007).

As expected an opposite trend was observed in the headspace, Fig. 4b shows the amount of released AITC increased in the lower fat content of the particles. This is in line with Dai and Lim (2014) who also observed a lower release rate of AITC from mustard seeds meal powder (MSMP) than from defatted MSMP. Another interesting implication of this effect of fat content is that the AITC from defatted particles was no longer detected in the headspace after 36 h, whereas the higher fat seeds maintained a certain level of AITC in the headspace (Fig. 4b). This prolonged remaining level of AITC is important to prolong the inhibiting effect on the spoilage bacteria in the package. As found by Liu and Yang (2010), AITC has more stability when the fat content of the seeds increased. Otherwise, when the particles contain low fat, AITC partitions into the water phase and headspace (Giroux et al., 2007). Hence, the fat content of particles strongly influence the partitioning of AITC between the particles and the headspace.

The stability of AITC in the headspace due to presence of fat can be optimal for microbial inhibition (Banerjee, Penna, & Variyar, 2015). In Fig. 4b, the presence of fat (2.8, 17.1, 29.1% fat) still retained AITC at around 1–2.5 µg/l until 48 h in the headspace. This AITC concentration is still below the MICs of AITC against the spoilage bacteria, e.g.

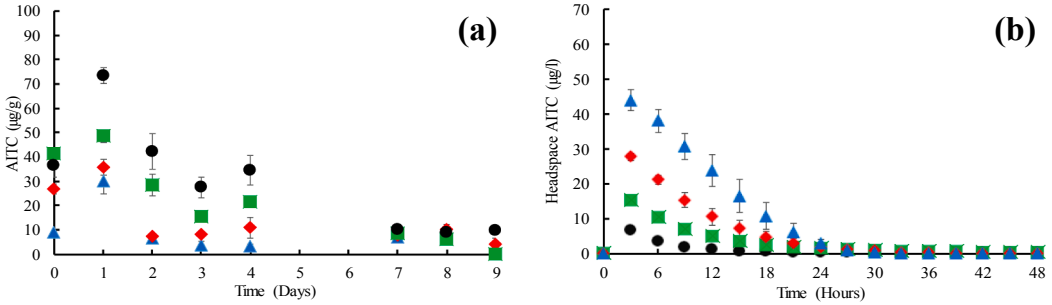

Fig. 4. The effect of fat contents : (▲) 0% fat; (♦) 2.8% fat; (■) 17.1% fat; and (●) 29.1% fat in the seeds 215–300 µm on (a) Allyl isothiocyanate (AITC) concentration in the seeds for 9 days, (b) headspace Allyl isothiocyanate concentration during 48 h.

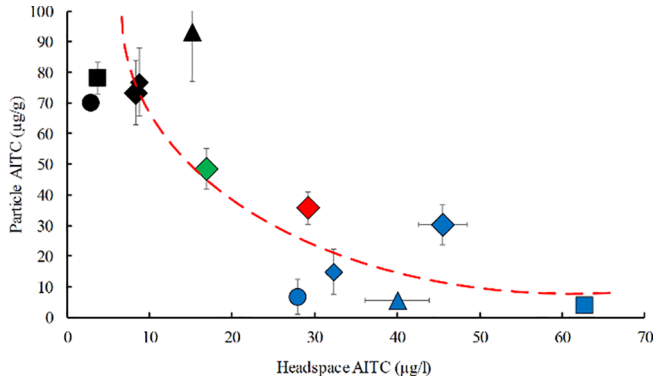

Fig. 5. The levels of headspace Allyl isothiocyanate (AITC) (peak concentration measured after 3 h) vs the levels of AITC in the particles (peak concentration measured after 1 day). Different shapes shows particle sizes; (▲) 50–100 µm; (♦) 200–315 µm; (■) 400–500 µm; and (●) 630–800 µm and different fat shows fat content; (♦) 0% fat; (♦) 2.8% fat; (♦) 17.1% fat; and (♦) 29.1%.

pseudomonas sp. (54 µg/l), yeast (16–22 µg/l), aflatoxin-producing fungi (10 µg/ml), and moulds (16–62 µg/ml) (Isshiki, Tokuoka, Mori, & Chiba, 1992), which means those bacteria is not sufficiently inhibited for 48 h. To reach sufficient microbial inhibition, the amount of ground mustard seeds added in the packaging system can be increased to produce a higher AITC release as observed by Torrijos, Nazareth, Perez, Manes, and Meca (2019) who reported that incorporation of 8 to 50 mg/g of oriental mustard flour into bioactive sauce increased the AITC concentration by 6 mg per liter packaging headspace. Otherwise, the microbial inhibition is expected to be higher initially in the absence of fat in the mustard particles as higher AITC was released into headspace. According to those aforementioned MICs, yeast and mould might be inhibited for 15–18 h before the bacteria start to quickly grow. From these results the usage of mustard particles without fat optimized release of AITC and the fat presence retains AITC in the headspace.

3.5. Correlation of AITC in the seeds with AITC in the headspace

Fig. 5 shows the concentration of AITC in the particles versus the concentration in the headspace for all fat contents and particle sizes. An inverse proportional trend is observed: particle sizes and fat contents that result in a lower particle AITC concentration give a higher headspace AITC concentration. The effect of the fat content is larger than that of the particle size in this respect. Reducing by 29% fat in the particles diminished AITC concentration around 40 µg per gram particles and increased almost the same amount per liter headspace. Reduction sizes from 630–800 to 50–100 µm increased less than 20 µg of AITC in the headspace and particles. This correlation reveals mass transfer of AITC from the particles to the headspace due to absence of fat in the particles.

3.6. The underlying mechanism of AITC formation and release

Based on the results obtained a mechanism for the AITC release kinetics was proposed as summarized in Fig. 6. The mechanism describes the formation of AITC upon cellular damage followed by the hydration of the particles that activates the formation of AITC from sinigrin by myrosinase. This formed AITC is subsequently partitioning into either the aqueous phase, fat phase, solid phase and the headspace. As AITC is not stable, its concentration will finally decrease in all

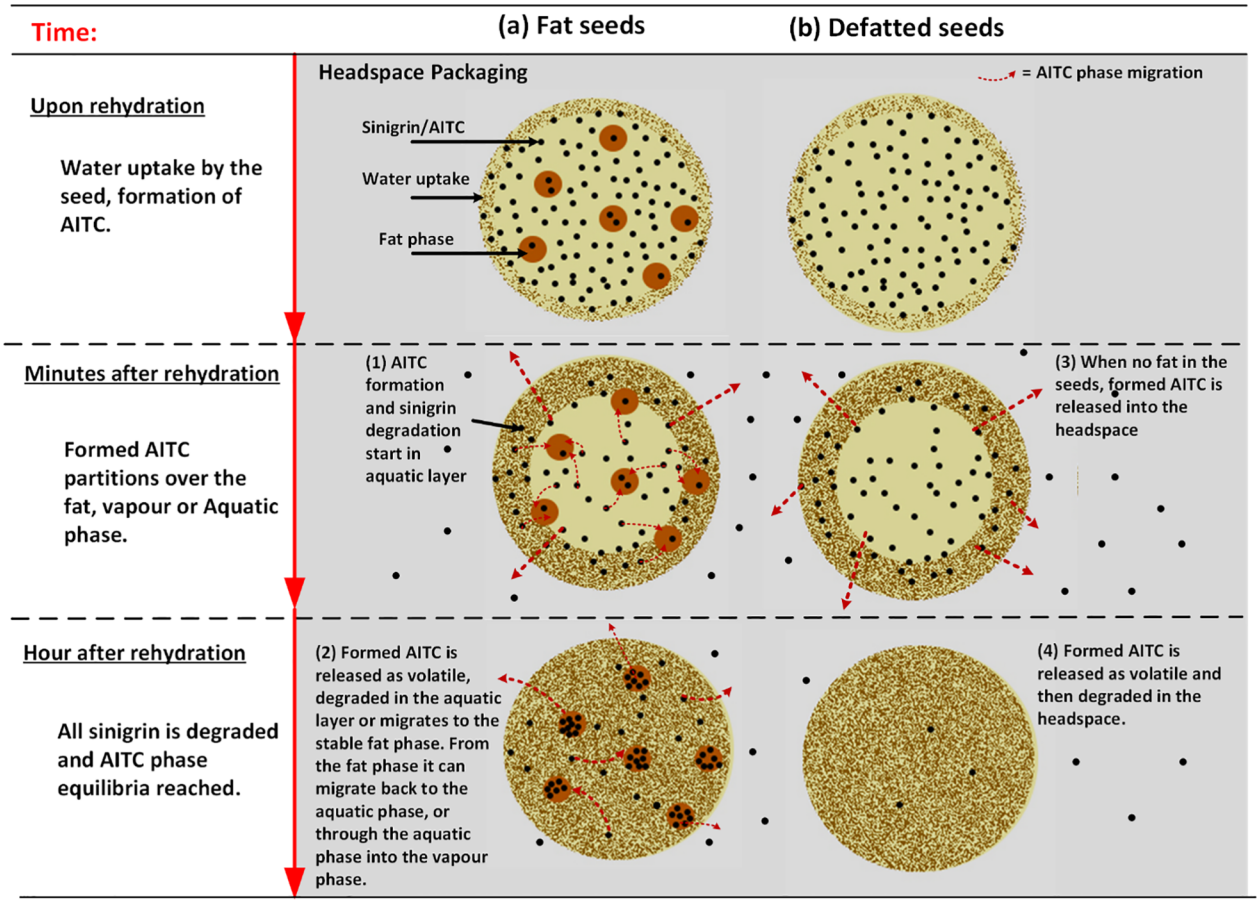

Fig. 6. The proposed molecular principle for Allyl isothiocyanate (AITC) formation and phase migration after hydration over time; (a) fat and (b) defatted mustard seeds.

phases. The stability is highest in the fat phase followed by the headspace and the aqueous phase.

This proposed mechanism describes the formation and partition of AITC over all phases. If fat is present, the majority of formed AITC migrates to this fat phase, thereby increasing the AITC concentration in the seeds and limiting the amount of released volatile AITC. Upon defatting, the fat phase in the seeds disappears, thereby decreasing the amount of AITC solubilized in the seeds and increasing the release of AITC to the headspace. Since the headspace concentration is relevant for microbial inhibition in packed food, the defatted ground mustard seeds induce a faster released of AITS in the headspace. However a slow and continuous release it is more desired and therefore it is useful to keep some fat in the seeds to act as a reservoir of stable AITC that can be released at a slow rate for an extended period of time. Further research is needed to create a model that could foresee the partitioning into different food matrices also taking into account the effect of external conditions like temperature or the nature of packaging material.

4. Conclusion

In this study, the effect of particle size and fat content of ground mustard seeds on sinigrin degradation, AITC formation, and AITC release into a packaging headspace was investigated. The concentration of AITC in the headspace is the result of the formation of AITC from sinigrin in the particles and the partitioning into the headspace. Sinigrin hydrolysis reaction was clearly influenced by sizes of the seeds and higher amount of sinigrin was degraded in the smaller ground seeds (50–100 μm). This higher degradation leads to a higher rate of released AITC in the headspace. The main factor influencing the concentration of AITC in the ground seeds was not the particle size but the fat content in the particles. This parameter in turn also influence the transfer of AITC into the headspace. Defatted seeds have the highest release of AITC in the headspace, while in the full fat the AITC was solubilized into the fat phase and their release into the headspace was much slower. The higher fat content resulted in an extended release period of the AITC from the seeds to prolong the time for its antimicrobial activity. These results support the design of an optimized active antimicrobial packaging by releasing AITC from the ground mustard seeds in order to inhibit the growth of microorganism.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125573.

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