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Stability and Antimicrobial Activity of Allyl Isothiocyanate during Long-Term Storage in an Oil-in-Water Emulsion

Tai-Ti Liu and Tsung-Shi Yang

Abstract: This study investigated the stability and antimicrobial activity of allyl isothiocyanate (AITC) in medium chain triglyceride (MCT) or soybean oil (SBO) dispersed in an oil-in-water (o/w) system during long-term storage. Oil type, content, and oxidative stability affect the stability and antimicrobial activity of AITC during storage. High oil content is favorable for AITC stability in the emulsion. Notably, AITC with MCT is more stable than AITC with SBO with the same oil content. Consequently, AITC with MCT is more effective than AITC with SBO in inhibiting G(−) bacteria (*E. coli* O157:H7, *Salmonella enterica*, and *Vibrio parahaemolyticus*) and G(+) bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*).

Keywords: allyl isothiocyanate, antimicrobial activity, medium chain triglyceride, oil-in-water emulsion, pathogens, soybean oil

Introduction

Allyl isothiocyanate (AITC), a naturally occurring compound, is present in the Cruciferae family. As a pungent flavor compound typically found in mustard and wasabi, AITC is generated from its precursor, allyl glucosinolate, namely, sinigrin (Kawakishi and Namiki 1969; Masuda and others 1996), which is widely available in seeds, roots, and leaves of many Cruciferae. When plant tissues are disrupted, sinigrin can be hydrolized to form AITC via myrosinase (thioglucoside glucohydrolase) activity (Chin and Lindsay 1993; Zrybko and others 1997). Generally, AITC can be used alone in, say, wasabi or in compound flavorings for processed foods which require such characteristic flavor. Additionally, AITC reportedly has antimicrobial activity against a wide range of microorganisms. For example, Ward and others (1998) investigated the inhibitory effects of horseradish distillates on *Staphylococcus aureus*, *E. coli* O157: H7, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Serratia grimesii*. Inatsu and others (2005) studied the antimicrobial activity of AITC and hop extract against *E. coli*, *Salmonella enteritidis*, *S. aureus*, *L. monocytogenes* and natural microflora in fermented Chinese cabbage.

Although it is advantageous to use AITC as a natural antimicrobial agent, its instability frequently limits its application in complex food systems. AITC is an electrophile, which is susceptible to the attack from nucleophiles such as water, OH^-, and amino groups; therefore, AITC is unstable in aqueous solution and easily decomposed, especially under alkaline conditions and elevated temperature (Ohta and others 1995). Several approaches have been developed to increase AITC stability. For instance, Ohta and others (2004) used cyclodextrins that included AITC as a complex. However, relatively strong physical interaction of flavor molecules and cyclodextrins may prove problematic for the release of flavor compounds (Padukka and others 2000). Chacon and others (2006) utilized gum acacia to microencapsulate AITC, and studied its effect on *E. coli* O157:H7 in refrigerated, nitrogen packed, finely chopped beef. One disadvantage of AITC in a powder form is that evenly distributing this powder in food systems is difficult; thus, AITC powder may be only suitable for certain foods to achieve antimicrobial efficiency.

When lipophilic and hydrophilic components are formulated to coexist in food systems, emulsions are typically required. Food emulsions cover a very wide range of practical applications such as sauces, dressings, butter, and various beverages. Therefore, it is very likely that AITC, if formulated, should be involved in food emulsion systems due to their ubiquity. Notably, AITC has been shown to have antimicrobial activity in a vapor or liquid state in many studies (Isshiki and others 1992; Lin and others 2000). However, little information about its antimicrobial effect in emulsions is available, especially for long-term storage. For instance, Inatsu and others (2005) used a commercial AITC-containing emulsion product "AIT-Hop" to control pathogenic or spoilage bacteria in lightly fermented Chinese cabbage; however, they did not reveal the emulsion composition.

Release of a volatile flavor compound such as AITC in an oil-in-water (o/w) emulsion is affected by the flavor compound partitioning among gas, oil, and water phases; thus, any factors influencing this partition may adversely affect its release and decomposition due to contact with water, especially in water dominant o/w emulsions. Therefore, the aims of this study were to investigate the effects of oil type, oil content, lipid oxidation, and emulsion stability on AITC decomposition in an o/w emulsion during long-term storage and to predict the effectiveness of the antimicrobial activity of AITC against pathogens during storage.

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Materials and Methods

Materials

The AITC, medium chain triglyceride (MCT), Tween 80 and Span 80 were purchased from Sigma-Aldrich Co. (St. Louis, Mo., U.S.A.). Soybean oil was obtained from Uni-Present Co (Tainan, Taiwan). Nutrition broth (Difco®), tryptic soy broth (TSB) and tryptic soy broth with 0.6% yeast extract (TSBYE) (Bacto™) were purchased from Becton Dickinson & Co. (San Jose, Calif., U.S.A.). *E. coli* O157:H7 (BCRC 14824), *Salmonella enterica* (BCRC 10746), *Vibrio parahaemolyticus* (BCRC 12863), *S. aureus* (BCRC 10781), *L. monocytogenes* (BCRC 10746) were acquired from Bioresource Collection and Research Center (BCRC) at the Food Industry Research and Development Inst. (Hsinchu, Taiwan).

Emulsion preparation

The AITC was first dissolved in MCT or SBO and both oils at 10% to 30% in the o/w emulsion were prepared. The final concentration of AITC in the emulsion was 2000 ppm (w/w). Emulsifiers Tween 80 and Span 80 were utilized to optimize the hydrophile-lipophile balance (HLB) value at 9 for the o/w emulsion preparation (2%, w/w), such that the emulsion could achieve a minimum average droplet size under a violent centrifugation (Orafidiya and Oladimeji 2002). A 10 mL aliquot of the o/w emulsion was poured into a 20-mL glass bottle, sealed with a cap with a septum inside, and then stored in a temperature-controlled chamber at 37 °C. All samples were prepared in duplicate.

Droplet size analysis

The oil droplet sizes in the emulsion were determined by a dynamic laser light scattering instrument (Microtrac S3000, York, Pa., U.S.A.). Droplet size distribution was expressed as a mean value in volume mode (mv) from 6 replicated determinations.

Turbidity measurement

In total, 10 mL of the o/w emulsion was withdrawn into a 20-mL glass bottle and sealed with a cap with a septum inside. The sample bottles were placed upside down and stored without disturbance at 37 °C. On each sampling day, 0.1 mL sample was gently withdrawn from the bottom (cap side) of the bottle using a 1-mL syringe with the needle tip piecing through the septum and reaching the sampling place marked on the outside of the bottle. Each sample was diluted with 5 mL of distilled water and the percentage transmission (%*T*) was determined at 600 nm with a spectrophotometer (Ultrospec 2100 Pro, Biochrom, Cambridge, U.K.). The turbidity of the diluted emulsion was calculated as follows: Turbidity = 100 − %*T* (Orafidiya and Oladimeji 2002). These values were the average of 3 determinations.

AITC analysis

The AITC content in the emulsion was determined by high-performance liquid chromatography (HPLC) (Shimadz, Japan) equipped with a UV detector set at 205 nm (Pechacek and others 1997). The solvent system was a mixture of acetonitrile and water (65 : 35, v/v). Isocratic elution was employed. A stainless steel column (C_{18}, 250 × 4.6 mm, Supecol, Bellefonte, Pa., U.S.A.) was utilized and the column flow rate was 1 mL/min. The AITC was quantitatively determined using a calibration curve established with the AITC standard under the same analytical conditions.

Peroxide analysis

An oil-containing emulsion in a sample bottle was frozen over night and melt at room temperature repeatedly for 3 cycles to destroy the emulsion into separated oil and aqueous phases. The oil layer was quantified and analyzed for hydroperoxides. The peroxide value was determined using the method (Cd 8–53) proposed by the American Oil Chemists' Society (AOCS).

Antimicrobial activity

Two to 4 loopfuls of bacteria (2 loopfuls for *E. coli* O157:H7, *V. Parahaemolyticus*, and *L. monocytogenes*; 4 loopfuls for *Salmonella enterica* and *S. aureus)* from stock cultures were inoculated in 100 mL nutrient broth (NB) and incubated at 37 °C for 16 h to increase the bacterial population to 2 to 6 × 10^8 (CFU/mL). The enriched cultures were diluted to approximately 10^5(CFU/mL) as an inoculant for subsequent tests. *E. coli* O157:H7*, Salmonella enterica*, and *S. aureus* were inoculated in the NB. *V. parahaemolyticus* and *L. monocytogenes* were inoculated in the TSB-3%NaCl and TSBYE, respectively. The inoculant (10%, v/v) was inoculated in the medium at various AITC emulsion concentrations (sterilized with a 0.2-μm syringe filter) and incubated at 37 °C overnight. Subsequently, the bacterial suspension was taken and diluted serially and a plate count was performed to determine the bacterial population via a dilution factor. Antimicrobial activity was expressed as an inhibition ratio calculated as follows: Inhibition ratio (%) = (l-$\log_{10}$ CFU/mL treated/$\log_{10}$ CFU/mL control) × 100%.

Statistical analysis

The o/w emulsion is a homogenous system; therefore, a completely randomized design was used. Statistical analysis of variance (ANOVA) was conducted using Statistica for Windows (StatSoft, Tulsa, Okla., U.S.A.). Tukey's HSD test was used to compare the mean values of data for significant difference at a 95% level.

Table 1–Calibration curves of microbial inhibition ratios compared with AITC concentrations.

AITC in oil/ bacteria treated	Inhibition ratio (%) range	Concentration range (ppm)	Regression equation	R^2
		MCT		
E. coli O157:H7	25 to 100	200 to 900	y = 9.4491x – 36.444	0.99
Salmonella enterica	10 to 100	5 to 100	y = 1.0624x – 12.158	0.94
S. aureus	30 to 100	200 to 2000	y = 25.266x – 670.7	0.96
L. monocytogenes	34 to 100	200 to 2000	y = 26.977x – 743.13	0.98
V. parahaemolyticus	9 to 100	10–50	y = 0.4481x + 3.6682	0.97
		SBO		
E. coli O157:H7	24 to 100	200 to 900	y = 9.1139x – 8.0679	0.99
Salmonella enterica	14 to 100	5 to 100	y = 1.0586x – 11.214	0.95
S. aureus	28 to 100	200 to 2000	y = 24.62x – 573.82	0.97
L. monocytogenes	30 to 100	200 to 2000	y = 25.033x – 558.28	0.98
V. parahaemolyticus	10 to 100	10 to 50	y = 0.4508x + 2.623	0.95

Table 2–Changes in oil droplet sizes in AITC o/w emulsions during storage at 37 °C.

Oil type and concentration	Droplet size (μm) Storage time (d)									
	0	10	17	24	31	38	59	94	139	180
MCT10%	1.26 ± 0.00[a]aA	1.26 ± 0.03aA	1.76 ± 0.31aA	1.19 ± 0.12aA	1.56 ± 0.37aA	1.26 ± 0.08aA	1.29 ± 0.10aA	1.26 ± 0.12aA	1.24 ± 0.14aA	1.26 ± 0.18aA
MCT20%	2.46 ± 0.02aB	2.46 ± 0.03aB	2.60 ± 0.02aB	2.29 ± 0.30aB	2.42 ± 0.02aB	2.44 ± 0.01 aB	2.45 ± 0.04aB	2.53 ± 0.04aB	2.61 ± 0.03aB	2.47 ± 0.01aB
MCT30%	2.96 ± 0.05aC	2.62 ± 0.00aB	3.03 ± 0.30aB	2.7 ± 0.30aB	2.89 ± 0.03aB	2.86 ± 0.01aC	2.86 ± 0.01aC	2.85 ± 0.01aC	2.84 ± 0.01aB	2.81 ± .04aBC
SBO10%	1.29 ± 0.06acA	1.24 ± 0.06aC	1.63 ± 0.02bA	1.31 ± 0.04acA	1.22 ± 0.01aA	1.24 ± 0.01acA	1.31 ± 0.01acA	1.34 ± 0.01acA	1.37 ± 0.01cA	1.35 ± 0.04acA
SBO20%	2.38 ± 0.02aD	2.60 ± 0.02abB	2.62 ± 0.07abB	2.58 ± 0.02abB	2.68 ± 0.06bB	3.05 ± 0.15cCD	2.66 ± 0.05bC	2.62 ± 0.04abB	2.58 ± 0.03abB	2.72 ± 0.10cB
SBO30%	3.61 ± 0.03aE	3.12 ± 0.26bD	3.21 ± 0.13aB	3.31 ± 0.01aBC	3.33 ± 0.09aBC	3.30 ± 0.07aD	3.36 ± 0.04aD	3.24 ± 0.01aD	3.12 ± 0.07bC	3.13 ± 0.06bC

[a]The values at the same row with different noncapitalized letters, and those at the same column with different capitalized letters are significantly different at $P \leq 0.05$.

Table 3–Changes in turbidity in AITC o/w emulsions during storage at 37 °C.

Oil type and concentration	Turbidity Storage time (d)									
	0	10	17	24	31	38	59	94	139	180
MCT10%	89.45 ± 0.07[a]aA	86.20 ± 0.22bA	85.11 ± 0.24bA	81.54 ± 0.60cA	81.29 ± 0.37cA	80.09 ± 0.06cA	65.28 ± 0.62dA	60.64 ± 0.64eA	59.50 ± 0.33efA	57.83 ± 0.69fA
MCT20%	98.96 ± 0.10aB	98.92 ± 0.10aB	95.34 ± 0.19cB	92.69 ± 1.17dB	88.20 ± 0.23eB	86.20 ± 0.18eB	69.09 ± 1.01fB	68.81 ± 0.61fB	67.90 ± 0.68fB	67.86 ± 0.42fB
MCT30%	99.41 ± 0.06aB	99.02 ± 0.03aB	96.64 ± 0.27bC	94.43 ± 0.01cB	89.03 ± 0.18dB	86.97 ± 0.21eB	66.88 ± 1.08fA	60.62 ± 0.62gAC	56.85 ± 0.70hAC	55.07 ± 0.37hC
SBO10%	95.07 ± 1.03aC	93.85 ± 0.43abC	90.19 ± 0.37bD	83.07 ± 1.21cA	81.46 ± 0.12cA	78.24 ± 0.53cC	63.68 ± 1.18dAD	60.78 ± 0.83deC	58.79 ± 0.67eC	53.73 ± 2.26fCD
SBO20%	98.80 ± 0.04aB	96.93 ± 0.35aD	92.24 ± 0.25cE	86.96 ± 0.21dC	83.21 ± 0.27eC	80.50 ± 0.63fA	74.76 ± 0.16gC	68.78 ± 0.57hB	69.09 ± 1.01hB	63.68 ± 1.18kB
SBO30%	99.18 ± 0.09aB	98.26 ± 0.06aB	93.08 ± 0.23bE	89.48 ± 0.27cC	83.93 ± 0.21dC	76.28 ± 0.39eE	61.09 ± 1.27fD	57.24 ± 0.14gD	53.21 ± 1.52hD	49.30 ± 0.83kD

[a]The values at the same row with different noncapitalized letters, and those at the same column with different capitalized letters are significantly different at $P \leq 0.05$.

Results and Discussion

Effects of oil type and amount on antimicrobial activity of AITC in an emulsion

Two types of oil MCT and SBO were used to assess the effects of oil type and amount on AITC antimicrobial activity in the emulsion. MCT is a saturated and relatively polar oil consisting of short carbon chains (C_8-C_{10}), whereas SBO is an unsaturated and relatively nonpolar oil with long carbon chains (mainly C_{18}). The MCT or SBO emulsion system without AITC was used as a control that did not have any significant antimicrobial activity (data not shown), indicating that AITC was the only active component in the emulsion system. However, the MCT or SBO may indirectly impact antimicrobial activity via physical or chemical interaction with AITC in the o/w emulsion system. The antimicrobial activity of AITC in the emulsion with the saturated oil (MCT) or the unsaturated (SBO) oil against the pathogens was assessed; antimicrobial activity was not significantly different ($P < 0.05$) for the 2 treatments due to the use of the same AITC concentration and oil type, but different amounts of oil during the microbial incubation period (data not shown). Therefore, the calibration curves of the inhibition ratios compared with AITC concentrations based on 20% oil content were used for subsequent comparisons of data for antimicrobial activity (Table 1).

The G(−) bacteria (*E. coli* O157:H7, *Salmonella enterica*, and *V. parahaemolyticus*) with minimal inhibitory concentrations (MICs) of 900, 100, and 50 ppm, respectively, were more sensitive to AITC in both oils than G(+) bacteria (*S. aureus* and *L. monocytogenes*) with MICs of 2000 ppm. The antimicrobial mechanism of AITC is likely due to its effects on cell membranes and on the leakage of cellular metabolites (Lin and others 2000). The G(+) and G(−) bacteria have different membrane structures (Salton 1963) and thus resulted in different permeability to AITC.

The trend of inhibition by AITC in the o/w emulsion against the G(+) and G(−) bacteria is similar to that obtained for AITC in vapor or liquid phase (Isshiki and others 1992; Lin and others 2000). For G(−) bacteria, the sensitivity to AITC inhibition was *V. parahaemolyticus* > *Salmonella enterica* > *E. coli* O157:H7 in terms of MIC. Although the 2 oils have different fatty acid saturation and relative polarity, analytical results demonstrate that oil type had no direct effect on the antimicrobial activity of AITC against the pathogens tested. The reason may be that the outer membrane of the G(−) bacteria lacks glycerophospholipids and, hence, lacks the effective channels for hydrophobic diffusion (Nikaido and Nakae 1979). The outer membrane of G(−) bacteria is resistant to neutral and anionic detergents (Vaara 1992). As MCT and SBO are neutral triglycerides, the degree of saturation of fatty acids in MCT or SBO did not influence AITC diffusion into the outer membrane of G(−) bacteria.

Emulsion stability during long-term storage

The degree of separation of an emulsion is a function of droplet size and size distribution, when all other factors are constant (Becher 1965). Because large droplets cream rapidly, small droplets should produce a more stable emulsion than large droplets. The degree of stability of an emulsion can be determined using the turbidimetric method, which measures the reduction in light transmitted directly through the emulsion, particularly through the creamed aqueous layer (Orafidiya and Oladimeji 2002). Therefore, emulsion stability during long-term storage was assessed by determining oil droplet size and turbidity.

The amount of oil is an important factor influencing emulsion droplet size through recoalescence because increasing the dispersed phase volume increases the number of oil droplets and collision frequency during homogenization (McClements 2004). Therefore, the initial droplet sizes in the emulsions containing the same type of oil increased when the amount of oil increased as shown in Table 2. When the droplet sizes of different oils used in the emulsions were compared, the droplet sizes of the MCT and SBO emulsions with the same amount of oil at a low oil content of 10% were initially similar after preparation, whereas the droplet size of the MCT emulsion was significantly smaller ($P \leq 0.05$) than that of the SBO emulsion at a high oil content of 30%. The reason is likely that the viscosity of MCT (data not shown) was lower than that of SBO; thus, MCT was easily to be broken into small droplets when a high amount of oil was used. However, the viscosity effect on droplet sizes was not significant at low oil content.

Except for emulsion droplet size with 20% SBO, which increased slightly, changes of droplet sizes for the other emulsions were not significantly different during storage for 180 d. This indicates that most of the oil droplets in the emulsions remained well dispersed or partly flocculated, but not coalesced.

In terms of turbidity, the change in turbidity for MCT emulsions with 10%, 20%, and 30% oil was approximately 35%, 31%, and 45%, respectively, during storage for 180 d, while that for SBO emulsions with 10%, 20%, and 30% oil was approximately 43%, 36%, and 50%, respectively (Table 3). Except for emulsions with 20% oil, the MCT emulsion was significantly more stable ($P \leq 0.05$) than the SBO emulsion for the same amount of oil during storage in terms of turbidity change. The decrease in turbidity in the emulsion with 20% oil was lowest among the 3 oil levels used, suggesting that this emulsion system was relatively stable compared with that of the 10% or 30% oil system. A high oil content can increase viscosity in an o/w emulsion (Giroux and others 2007), promoting the stability of an emulsion in terms of viscosity. However, oil droplet size also increased in this study, which adversely impact emulsion stability. Conversely, low oil content generated small droplets and reduced viscosity. Therefore, the relative stable emulsion with 20% oil may be ascribed to the balance between viscosity and droplet size.

Table 4–Changes in AITC content in o/w emulsions during storage at 37 °C.

Oil type and concentration	Decrease ratio (%) Storage time (d) 0	10	17	24	31	38	59	94	139	180
MCT10%	0 ± 0[a]aA	8 ± 0bA	12 ± 0bA	29 ± 4cA	33 ± 1cA	40 ± 1dA	59 ± 0eA	60 ± 1eA	68 ± 2fA	80 ± 2gA
MCT20%	0 ± 0aA	3 ± 1aA	3 ± 1aB	12 ± 1bB	18 ± 1bcB	24 ± 2cA	50 ± 3dAC	55 ± 1deAD	57 ± 1eB	66 ± 1fB
MCT30%	0 ± 0aA	2 ± 1aA	2 ± 1aB	5 ± 1abC	7 ± 1abC	12 ± 1bcB	14 ± 4cB	14 ± 4cB	30 ± 1dC	42 ± 2fC
SBO10%	0 ± 0aA	19 ± 5bB	23 ± 2bcC	29 ± 1cA	42 ± 0dD	47 ± 2dC	59 ± 1fA	70 ± 4gC	74 ± 2gD	88 ± 1hD
SBO20%	0 ± 0aA	11 ± 1bB	10 ± 1bAD	15 ± 2bB	23 ± 1cB	28 ± 0dD	53 ± 0eC	56 ± 1eD	59 ± 1fB	68 ± 1gB
SBO30%	0 ± 0aA	0 ± 0aA	7 ± 0abD	11 ± 2bB	15 ± 3bB	19 ± 2bcE	24 ± 4cB	33 ± 1dE	43 ± 0eE	63 ± 1fB

[a]The values at the same row with different noncapitalized letters, and those at the same column with different capitalized letters are significantly different at $P \leq 0.05$.

AITC stability during long-term storage

As a volatile flavor compound, the release of AITC in an o/w emulsion is determined by its partition among gas, oil, and water phases; thus, any factors influencing its partition in an emulsion may affect its release and decomposition due to contact with the water phase, especially in water dominant o/w emulsions. Factors such as oil droplet size, oil content, and oil type affect the release of volatile compounds in an emulsion system (Rabe and others 2003a; Roberts and others 2003). When the same oil type (MCT or SBO) was used in the AITC o/w emulsion, AITC stability increased as oil content increased (Table 4). In terms of MCT, the decreased quantity ratios for AITC with 10%, 20%, and 30% oil were approximately 80%, 66%, and 42%, respectively at day 180. In contrast, those of AITC with 10%, 20%, and 30% SBO were approximately 88%, 68%, and 63%, respectively, suggesting that at a low oil content, more AITC was partitioned into the water phase than the oil phase because less oil volume was available for the same amount of AITC in the emulsion. That is, the water content relatively increased, which was unfavorable for AITC stability. The rate of flavor release is strongly related to oil concentration, with oil phase acting as an aroma reservoir. Flavor release becomes slow as the oil fraction of the emulsion increases (Giroux and others 2007).

When different oils used in the emulsion were compared, we found that AITC with MCT generally degraded more slowly than that with the SBO at the same oil content (except for 20%) during storage (Table 4). Structurally, AITC has a polar isothiocyanate end and a nonpolar allyl side chain. This structure is similar to that of an amphiphilic compound. However, AITC is naturally oil soluble, meaning that its chemical hydrophobicity is greater than its hydrophilicity. One theory argues that the chain length of the fatty acids influences the absorption of volatile compounds, where longer chains increase the absorption effect (Bakker 1995). Because SBO has fatty acids with longer chains than MCT, SBO is more hydrophobic than MCT. Theoretically, SBO should have higher affinity to AITC than MCT and retain more AITC via the hydrophobicity effect. Conversely, another study suggested that lipid molarity is important to the release of volatile compounds. When the same amount of oil is used in an emulsion, the oil with a low molecular weight (MW) has a higher molarity than that with a high MW. A high molarity means that the number of molecules is large; therefore, MCT (low MW) should have more molecules than SBO (high MW) in the emulsion. The large number of lipid particles should lead to an increased number of lipid-flavor interactions (Rabe and others 2003b), thereby effectively retaining flavor compounds.

In this study, both hydrophilicity and molarity may simultaneously affect the release of AITC in the emulsion; the magnitudes of these influences depend on oil content. At low oil content, say, 10%, the molarity effect was more dominant and, consequently, AITC in MCT was more stable than that in SBO. When oil content was 20%, both hydrophilicity and molarity effects were balanced and, thus, no significant difference existed in AITC stability between the 2 oils. However, at 30% oil, AITC with MCT was again more stable than that with SBO. This phenomenon may result from the different oxidative stability of the oils. The peroxide value (POV) of MCT was undetectable in this study; therefore, MCT was more stable than SBO in terms of oxidative stability as indicated by the POV (Figure 1). The most probable factor altering the chemical property of the oils used in experiments was lipid oxidation. Notably, SBO contains abundant polyunsaturated fatty acids that are susceptible to oxidation, of which the hydroperoxides and derived free radicals may further accelerate the decomposition of AITC. As oil content increases, the POV in the SBO increases (Figure 1). Both oil content and oxidative stability affected AITC stability; the former had a greater effect than the latter. Evidently, less AITC in the 30% SBO was lost than that in the 10% MCT at day 180 (Table 4), even though the POV in 30% SBO was higher than that in the 10% MCT (Figure 1). However, at the same oil content, the increase in POV is unfavorable for AITC stability in the emulsion. For example, the AITC with 30% SBO decomposed spontaneously approximately 10% excluding the lipid oxidation effect, as indicated by insignificant POV variation ($P > 0.05$) during storage for 45 d (day 94 to 139) (Figure 1). Whereas, the AITC with 30% SBO decreased by approximately 23%, of which 13% was ascribed to the effect of increased POV (from 10 to 14 meq/kg) between day 139 to 180 (51 d).

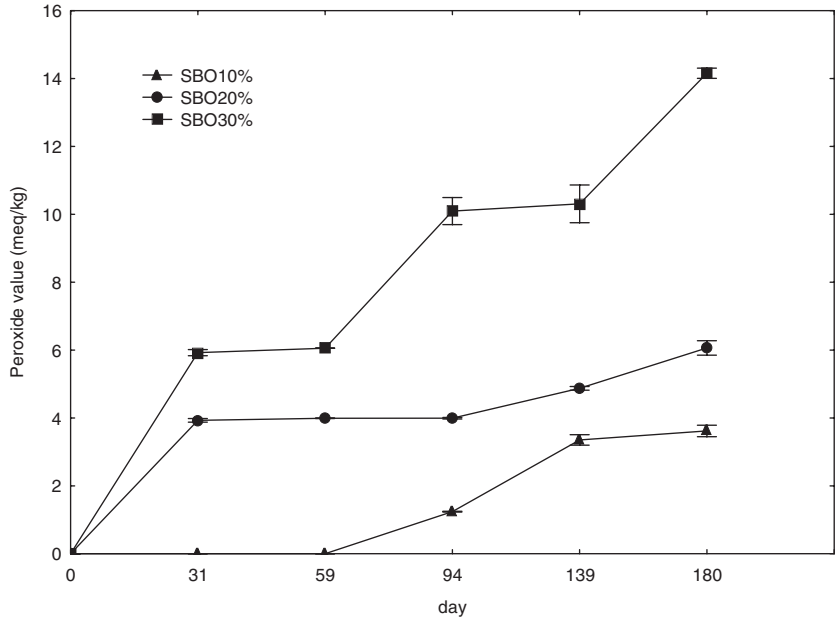

Figure 1–Changes in peroxide values of AITC-containing soybean oil in AITC o/w emulsions during storage at 37° C.

Table 5–Microbial inhibition in AITC o/w emulsions during storage at 37 °C.

Bacteria	Oil type and concentration	Inhibition ratio (%) Storage time (d) 0	10	17	24	31	38	59	94	139	180
E. coli O157:H7	MCT10%	100 ± 0[a]aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	86 ± 0bA	84 ± 1bA	69 ± 4cA	45 ± 5dA
	MCT20%	100 ± 0[b]aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aB	95 ± 1bB	91 ± 2cB	72 ± 2dB
	MCT30%	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aB	100 ± 0aC	100 ± 0aC	100 ± 0aC
	SBO10%	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	86 ± 2bA	68 ± 1cD	55 ± 4dD	27 ± 2eD
	SBO20%	100 ± 0aA	100 ± 0 aA	100 ± 0 aA	100 ± 0 aA	100 ± 0 aA	100 ± 0 aA	100 ± 0aB	97 ± 1aB	90 ± 2bB	73 ± 4cB
	SBO30%	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aB	100 ± 0aC	100 ± 0aC	80 ± 1bB
Salmonella enterica	MCT10%	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA
	MCT20%	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA
	MCT30%	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA
	SBO10%	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA
	SBO20%	100 ± 0aA	100 ± 0aA	100 ± aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA
	SBO30%	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA
V. parahaemolyticus	MCT10%	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA
	MCT20%	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA
	MCT30%	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA
	SBO10%	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA
	SBO20%	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA
	SBO30%	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA	100 ± 0aA
S. aureus	MCT10%	100 ± 0aA	95 ± 0bA	92 ± 0bA	80 ± 3cA	76 ± 1cA	71 ± 1dA	57 ± 0eA	56 ± 0eA	50 ± 1fA	42 ± 2gA
	MCT20%	100 ± 0aA	100 ± 0aA	100 ± 0aB	93 ± 1bB	88 ± 1cdBF	84 ± 1dBF	64 ± 2eB	61 ± 0eA	59 ± 1eB	52 ± 1fBE
	MCT30%	100 ± 0aA	100 ± 0aA	100 ± 0aB	100 ± 0aC	99 ± 1aC	95 ± 1bC	95 ± 0bC	91 ± 1cB	81 ± 1dC	72 ± 2eC
	SBO10%	99 ± 1aA	85 ± 4bB	82 ± 1bC	77 ± 1cD	67 ± 0dD	64 ± 2dD	55 ± 1eA	46 ± 3fC	44 ± 2fD	33 ± 1gD
	SBO20%	100 ± 0aA	94 ± 1bA	95 ± 1bAD	91 ± 1bB	85 ± 1cEB	80 ± 0dEB	61 ± 0eB	59 ± 1eD	56 ± 1fB	50 ± 2gE
	SBO30%	100 ± 0aA	100 ± 0aA	97 ± 0aD	94 ± 1bB	91 ± 2bcF	88 ± 2cF	84 ± 3cD	77 ± 1eE	68 ± 0fE	52 ± 0gE
L. monocytogenes	MCT10%	97 ± 1aA	92 ± 0bA	89 ± 0bA	78 ± 3cA	74 ± 1cA	69 ± 1dA	56 ± 0eA	56 ± 0eA	50 ± 1fA	42 ± 2gAD
	MCT20%	100 ± 0aA	95 ± 3bA	96 ± 1abB	89 ± 1cB	85 ± 1cBC	73 ± 1dcA	63 ± 2eA	60 ± 0efAD	58 ± 1fB	51 ± 1gB
	MCT30%	100 ± 0aA	99 ± 0aA	99 ± 0aB	97 ± 0aC	95 ± 1abC	92 ± 1bcB	90 ± 2bcB	90 ± 3cB	78 ± 0dC	70 ± 2eC
	SBO10%	97 ± 1aA	83 ± 4bB	80 ± 1bC	75 ± 1bD	65 ± 0cD	62 ± 2cC	53 ± 1dC	45 ± 3deC	42 ± 2efD	34 ± 4fD
	SBO20%	100 ± 0aA	92 ± 1bA	92 ± 1bD	89 ± 1bB	83 ± 1cB	78 ± 0dD	66 ± 1eA	64 ± 1eD	55 ± 1fB	48 ± 2gAB
	SBO30%	100 ± 0aA	100 ± 0aC	95 ± 0abD	92 ± 1bcB	89 ± 2cdC	86 ± 2deE	82 ± 3eD	75 ± 0fE	67 ± 0gE	51 ± 0hB

[a]The values at the same bacterium and row with different noncapitalized letters, and those at the same bacterium and column with different capitalized letters are significantly different at $P \leq 0.05$.
[b]No visible microbial colonies found in plate counting is defined as 100% inhibition.

Additionally, another reason why AITC with 10% MCT or SBO has higher decrease ratio than that with 20% and 30% MCT or SBO may be partly due to smaller oil droplet sizes compared with those of AITC with 20% and 30% MCT or SBO. Small oil droplets in the emulsion may lead to accelerated mass transfer due to the increased interfacial area and the shortened diffusion distance through the oil droplets (Rabe and others 2003a).

Antimicrobial activity of AITC during long-term storage

The antimicrobial activity of AITC with MCT or SBO at different oil levels during long-term storage was predicted based on calibration curves of inhibition ratios of the pathogens compared with the AITC concentrations (Table 1). Table 5 shows the antimicrobial activity of AITC on the pathogens during storage. In terms of antimicrobial activity on G (−) bacteria, *Salmonella enterica*, and *V. parahaemolyticus* were completely inhibited during storage for 180 d, while the antimicrobial activity for *E. coli* O157:H7 was not 100%, but depended on the oil type and content after the same storage period. Before day 38, AITC with MCT or SBO at any oil level achieved 100% inhibition on *E. coli* O157:H7. However, for AITC with MCT at 10%, 20%, and 30%, the inhibition ratios were 45%, 72%, and 100%, respectively, at day 180. This analytical finding indicates that at least 30% MCT is required to maintain a sufficient amount of AITC for complete inhibition of *E. coli* O157:H7. In contrast, the inhibition ratios were 27%, 73%, and 80% for AITC with SBO at 10%, 20%, and 30% oil at day 180, respectively. Consequently, even 30% SBO did not completely inhibit *E. coli* O157:H7. The experimental results show that the inhibitory effect of AITC with MCT is comparable with that with SBO at 20%; however, the former is better than the latter at 10% or 30% oil content.

Similarly, the inhibition of AITC on G(+) bacteria (*S. aureus* and *L. monocytogenes*) also depended on oil type and content during storage. However, the general inhibitory effects were inferior to those on G(−) bacteria. In terms of the inhibition of G(+) bacteria, over 95% inhibition was achieved using AITC with 30% MCT at storage for approximately 1 mo. The MCT or SBO at all oil levels achieved at least 50% inhibition at storage for approximately 2 mo. However, as storage time increased, inhibition ratios decreased to approximately 42%, 52%, and 72% for AITC with MCT at 10%, 20%, and 30%, respectively, at day 180, whereas the inhibition ratios were approximately 33%, 50%, and 52% for AITC with SBO at 10%, 20%, and 30%, respectively, for the same period. The experimental results show that AITC with 30% MCT was more effective than that with 30% SBO in inhibiting G(+) bacteria because the stability of AITC with 30% MCT was higher than that with 30% SBO during storage for 180 d.

Conclusions

The oil type, content, and oxidative stability affected AITC stability and antimicrobial activity in the o/w system during long-term storage. The emulsion system was relatively stable during long-term storage, as shown by the changes in oil droplet size and turbidity. This analytical finding suggests that changes in the physical environment may not have major effects on the stability of AITC during storage. Rather, the chemical environment such as hydrophilicity, molarity, and the oxidative stability may have relatively greater effects on AITC stability. Notably, MCT was better than SBO in stabilizing AITC in the o/w emulsion. Additionally, AITC was more effective in inhibiting G(−) bacteria than on G(+) bacteria in the emulsion during storage. To determine emulsion stability, the temperature used in this study was relatively higher than that associated with general storage conditions (for example, room temperature or refrigeration at 4 to 10 °C). Therefore, the AITC stability was expected to markedly higher in those general conditions than that in this experimental condition, and its antimicrobial activity was proportionally enhanced. Advantageously, storage time for AITC-containing emulsions can be extended in the general storage conditions. Notably, SBO is widely used in numerous food products, and its oxidative stability can affect AITC stability and antimicrobial activity. Information provided by this study will prove useful for food product developers interested in using AITC as a flavor or natural antimicrobial agent in o/w emulsion food systems.

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