

Development of Encapsulated Wasabi Flavor for Resistance in High Temperature Condition

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Abstract

The present work was aimed to develop encapsulated wasabi flavor beads for resistance high temperature condition. The beads prepared from waxy maize starches (HI-CAP 100), modified tapioca starches (Flavotec), alginate and chitosan at various concentrations to determine their properties. The beads size was found not significant differences in all treatments. Microcapsules of 10 % wasabi flavor derived from the mixture of HI-CAP 100 and Flavotec at mass ratio of 1:0 (w/w) and alginate and chitosan at mass ratio of 14:1 (w/w) exhibited excellent encapsulation efficiency (96.42 %) and this formulation is also demonstrated the highest retention of AITC in wasabi flavor beads after thermal process. Therefore, the encapsulated wasabi flavor has broad application prospects in food industry and development value prospects.

Keywords: Wasabi, Allyl isothiocyanate, Flavor, Encapsulation, High temperature condition

Introduction

The flavor of a dish determines the choice of spices used in cooking. Wasabi (*Wasabia japonica Matsum*) is one of condiment whose increased consumption worldwide is attributable to their characteristic flavor. It can be used to decorate foods because of its bright green color and also to flavor traditional and modern foods e.g. raw fish, noodles, sushi, and mayonnaise [1]. More recently, wasabi has found widespread appeal in western cuisine, as well as in Japanese cuisine, owing to its unique hot taste and pungent smell. The main pungent component of wasabi is known to be isothiocyanate (ITC) and its derivatives, volatile substances, and allyl isothiocyanate (AITC) account for more than half ITC derivatives [2]. When the cells are disrupted during preparation, the glucosinolates reacts with the membrane-associated myrosinases liberating isothiocyanates [3]. The highest tissue concentration of AITC was found in the rhizomes, ranging from 1564 to 3366 mg/kg (fresh weight basis) [4]. However, it has been shown that AITC is unstable and is gradually decomposed to other compounds having a garlic-like odor in the presence of water at both room temperature (25 °C) and 37 °C [5]. AITC is also reported to be sensitive by several hindrances, including instability at high temperature, poor solubility, and susceptibility to degradation by food nucleophiles such as amino acids, amines, proteins, sulfites and alcohols [6]. Encapsulation process which is an effective method to prevent the sensitive ingredients within wall materials from adverse environment is therefore applied to tackle such problems.

The effective wall materials should be based on good emulsification, low viscosity at high concentrations, good dissolution and network-forming characteristics as well as the ability to control the flavor release profile in certain mediums [7]. Aside from the wall materials, the interactions between flavor compounds and wall materials may also affect the flavor retention such as molecular association of the flavor compounds with wall material through hydrogen bonding and development of insoluble complexes [8]. Previous reports showed that low molecular weight aroma compounds (i.e. allyl isothiocyanate) could be binding to native starch demonstrated good retention of flavor when compared to pure aroma [9]. Several encapsulation methods for protection of AITC against degradation have been mostly employed in powder forms. The AITC was entrapped by inclusion in α - and β -cyclodextrins complexes and their interaction were investigated [10-13]. These studies reported that AITC less than 20% remained in 48 h. In another study, the encapsulated AITC prepared by spray drying after emulsification using different mass ratios of gum arabic with Tween 20 or Span 80 was released

approximately 20 % from the microcapsules during 15 days of storage at 25 °C and 50 % relative humidity [14]. In the past few years, AITC encapsulation has been applied for use in the field of food packaging due to antimicrobial properties against foodborne pathogens. The encapsulation of AITC (4 %) using zein ultrafine fibers was efficient to maintain strawberry quality under refrigeration [15]. The LDPE films containing encapsulated AITC in triacetyl- β -cyclodextrin maintained consistent AITC release, this evidence suggests that it can be applied as an effective antimicrobial packaging material for food and nonfood applications [16]. In the group of biopolymers, waxy maize starches are one of the most preferred wall materials due to their good emulsifying and film forming properties, low viscosities, high oil loading capacities, oxygen barrier properties, low molecular weight and low in price [17]. Additionally, the unique advantages of waxy maize modified starches are that they work as stabilizers of nanoemulsions and then serve as wall materials. The modified tapioca starch is widely used in the food industry to contain and protect volatile compounds, acting as containment materials for encapsulating aromas and stabilizing emulsions. Loksuan [18] studied ability of modified tapioca starch to serve as wall materials for encapsulating β -carotene. They reported that the total β -carotene was highest for modified tapioca starch while it was lowest for maltodextrin. Results obtained suggest that the modified tapioca starch can be considered as potential wall material for encapsulation of β -carotene. Nevertheless, there are very few researches about the utilization modified starch for encapsulation of AITC and wasabi flavor. A gel bead system based on natural polysaccharides consisted of alginate and chitosan has been widely used for encapsulation different bioactive ingredients due to their simple use, low cost, biocompatibility and biodegradability which could be employed as an effective matrix. The chitosan–calcium alginate complex gel beads showed the highest bead stability and AITC retention under simulated gastrointestinal pH conditions [19]. According to Pasparakis and Bouropoulos [20], coating of the alginate beads with chitosan caused significant reduction of micro/macroscale pores and cracks observed on the surface and thus a decrease of its permeability. However, very few studies have been reported on the AITC-entrapped polysaccharide gel bead system. Also, many food systems demand the application of AITC under thermal processing, but little studies dealt with AITC stabilization as a flavoring ingredient for thermal processed foods.

To the best of our knowledge, no study has been performed to encapsulate wasabi flavor and its application under thermal process (microwave heating and autoclaving), therefore this study was designed to develop encapsulated wasabi flavor with waxy maize starch (HI-CAP 100), modified tapioca starch (Flavotec), alginate and chitosan at various concentrations for resistance in high temperature condition.

Materials and methods

Materials

Chemical: Wasabi flavor was obtained from V. Mane Fils (Bangkok, Thailand), octenyl succinylated waxy maize starches (HI-CAP 100) from National Starch and Chemical (Bangkok, Thailand), modified tapioca starches (Flavotec) from Siam Modified Starch (Bangkok, Thailand), alginate from Union Chemical (Bangkok, Thailand), chitosan from Marine Bio Resources (Samutsakhon, Thailand), allyl isothiocyanate (AITC) with 98 % GC purity from Fluka Chemical Company (Steinheim, Germany). All other reagents used in this study were of analytical grade and commercially available.

Production of encapsulated wasabi flavor in beads

The wall material solution was prepared by dissolving powdered ingredients in distilled water. The production of wasabi flavor beads was divided into 2 steps: 1) blending wasabi flavor (10 % of total solids) with HI-CAP 100 and Flavotec solution at mass ratio of 0:1, 1:1 and 1:0 (w/w) under warm water for 5 min, 2) adding of alginate (3 % w/w) and chitosan (2 % w/w) solution at mass ratio of 10:1, 12:1 and 14:1 (w/w) and mixed using homogenizer at a rotational speed of 6,700 rpm for 45 min. The mixture was dispersed as droplets using a syringe into a beaker containing calcium chloride solution (2 % w/w) and then stirred for 30 min that solidified the beads. There were 9 different formulations (D1, D2, D3, D4, D5, D6, D7, D8, D9) used for encapsulation in **Table 1**. The beads were then collected by filtration and subsequently washed with distilled water and kept in amber bottles and stored at 4 °C. The encapsulation process was carried out in triplicate.

Table 1 Wall materials composition for wasabi flavor encapsulation.

| Treatments | Mass ratio (w/w) | | | |
|------------|------------------|--------------|--------------|--------------|
| | Step 1 | | Step 2 | |
| | HI-CAP 100 (H) | Flavotec (F) | Alginate (A) | Chitosan (C) |
| D1 | 0 | 1 | 10 | 1 |
| D2 | 0 | 1 | 12 | 1 |
| D3 | 0 | 1 | 14 | 1 |
| D4 | 1 | 0 | 10 | 1 |
| D5 | 1 | 0 | 12 | 1 |
| D6 | 1 | 0 | 14 | 1 |
| D7 | 1 | 1 | 10 | 1 |
| D8 | 1 | 1 | 12 | 1 |
| D9 | 1 | 1 | 14 | 1 |

Characterization of wasabi flavor beads

The characterization of the beads was investigated by determining properties consisted of diameter, weight, beads yield percentage, total AITC in encapsulated wasabi flavor and encapsulation efficiency. All the tests were performed in triplicate.

The size of beads

The size of beads was measured with calipers. A diameter of 50 beads was determined to calculate the average diameter. The mean of diameter (D) was calculated using Eq. (1):

$$D = \sum \frac{d}{n} \quad (1)$$

where d is the diameter of each beads, n is the total number of beads measured, and D is the mean diameter.

The weight of beads

The weight of 50 beads was determined with 2-digit precision balance (Sartorius BSA2202S, Germany) and express as the average weight.

Beads yield percentage

The beads yield percentage was determined according to the following Eq. (2) [21]:

$$\text{Beads yield (\%)} = (A/A_i) \times 100 \quad (2)$$

where A is amount of recovered beads, A_i is amount of emulsion in initially used

Determination of total AITC in wasabi flavor

Total AITC in the encapsulated wasabi flavor was measured according to solid phase microextraction (SPME) a modified method described by Tomson *et al.* [22]. The 10 g of wasabi flavor-loaded beads were placed in a 40-mL headspace glass vial and kept on the hot plate at 40 °C for 15 min to equilibrate. The volatiles were extracted with the fibers (PDMS/DVB) (Supelco, Bellefonte, PA, USA) at 40 °C for 30 min. All volatile samples were analyzed by GC-7890A (Agilent, USA) which was equipped with a DB-WAX capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) and flame ionization detector. The injector and detector temperatures were set at 160 and 240 °C, respectively. Column temperature started at 100 °C, held for 2 min, and then increased at 10 °C/min to 145 °C for 5 min, followed by 10 °C/min to 230 °C for 5 min. Nitrogen was used as the carrier gas at a constant flow rate. The peak areas of volatile compounds were obtained from the chromatograph software. Quantification of allyl isothiocyanate was performed using methyl isothiocyanate as an internal standard.

Determination of encapsulation efficiency

The formula used for calculating encapsulation efficiency for AITC is given below in Eq. (3) [21]:

$$EE\% = (C/C_i) \times 100 \quad (3)$$

where C is concentration of AITC in encapsulation beads, C_i is initial concentration of AITC in solution.

Effect of thermal processing on encapsulated wasabi flavor

Microwave heating and autoclaving were the model system chosen for study the resistance high temperature of encapsulated wasabi flavor. The 5 g of wasabi flavor-loaded beads and 50 mL of water were heated in a microwave oven at power of 1,000 W for 28 s. For autoclaving, the encapsulated wasabi flavor was mixed into the tuna spread (50 % mayonnaise, 25 % minced water chestnut, 20 % tuna and 5 % chopped onions). The 5 g of wasabi flavor-loaded beads and 85 g of tuna spread were weighted and contained in enamel-coated cans (211×109). The cans were sealed and heated in a vertical autoclave at 121 °C for 30 min and then allowed to cool for 15 min. The retaining wasabi flavor (total AITC) and encapsulation efficiency were determined and calculated as described in section 2.3.4 and 2.3.5, respectively.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) to determine differences between samples from 3 replicate experiments followed by Duncan's multiple range test ($p < 0.05$) to identify significant differences among treatments, using the statistical software SPSS (ver. 23, IBM, Armonk, NY, USA).

Results and discussion

Characterization of wasabi flavor beads

The physical properties of wasabi flavor beads made from different wall materials with various concentrations are shown in **Table 2**. There was no significant difference ($p > 0.05$) in size of the beads from all treatments which had an average size in the range of 3.16 ± 0.02 to 3.70 ± 0.02 mm, resulted in uniform beads, while that of weight were significantly different ($p < 0.05$). The average weight of beads is in the range of 41.33 ± 1.05 to 59.99 ± 1.19 mg, it was found to increase with increase in concentration as well as viscosity of alginate solution [23]. The present study results were consistent with the results of Taksima *et al.* [24]. They had reported similar results for the diameter and weight of alginate-chitosan coated beads using ultrasonic atomizer for astaxanthin. The mean diameter and weight of alginate-chitosan beads were 4.23 mm and 55.47 mg, respectively.

With respect to the beads yield percentage, it was found to be in the range of 61.70 to 89.91 %. The yield (%) of all 9 treatments showed that an increase in the concentration of the alginate increases the practical yield percentage. This result is supported by Aldawsari *et al.* [25] who reported that the enhanced ratio of alginate in the composition of the beads forms a more rigid and the bead yield became higher when the alginate concentration was increased.

The encapsulation efficiency (EE) is important indicator to evaluate the success of encapsulation, which is defined as the amount of active ingredients encapsulated inside the beads. In our study, EE values of AITC in various concentrations of wall materials are shown in **Figure 1**. The initial EE was determined after the encapsulation process (day 0) and expressed as the percentage of encapsulated AITC relative to the total AITC used. The data indicated that all treatments differed significantly and ranged from 63 to 96 %. D6 had maximum EE (96.42 %), wall material consisted of HI-CAP 100 and Flavotec at mass ratio of 1:0 (w/w) and alginate and chitosan at mass ratio of 14:1 (w/w). The beads prepared with HI-CAP 100 presented the best encapsulation efficiency values. This may be related to the effect of emulsifying property of HI-CAP 100 that exhibits lipophilic groups on its structure which is capable of adsorbing to an oil-water interface resulting in molecular association of the wall material with flavor compounds through hydrogen bonding, may also affect the flavor retention [8]. The results were similar to those of Carvalho *et al.* [26] who reported the EE of green coffee oil in modified starch HI-CAP 100 microparticles up to 97 %. Ratanasiriwat *et al.* [27] also reported microcapsules of 20 % wasabi flavor derived from the mixture of HI-CAP 100 with maltodextrin and HI-CAP 100 with CAPSUL demonstrated excellent properties of EE. Furthermore, this result demonstrated that the increase of alginate concentration enhanced flavor loading efficiency could be due to more availability of the calcium-binding sites in the anionic linear polysaccharide chain and, consequently, an additional degree of crosslinking by increasing the sodium alginate fraction [28,29]. The enhancement of alginate concentration resulted in increase of EE value was confirmed in the study of Lotfipour *et al.* [30] who indicated that alginate concentration was the most affective factor on EE of propranolol and increase in EE of *Lactobacillus acidophilus* incorporated into calcium alginate beads [31]. In addition, the electrostatic interaction between chitosan and alginate might render the network structure of chitosan-coated calcium alginate gel beads more entangled [20].

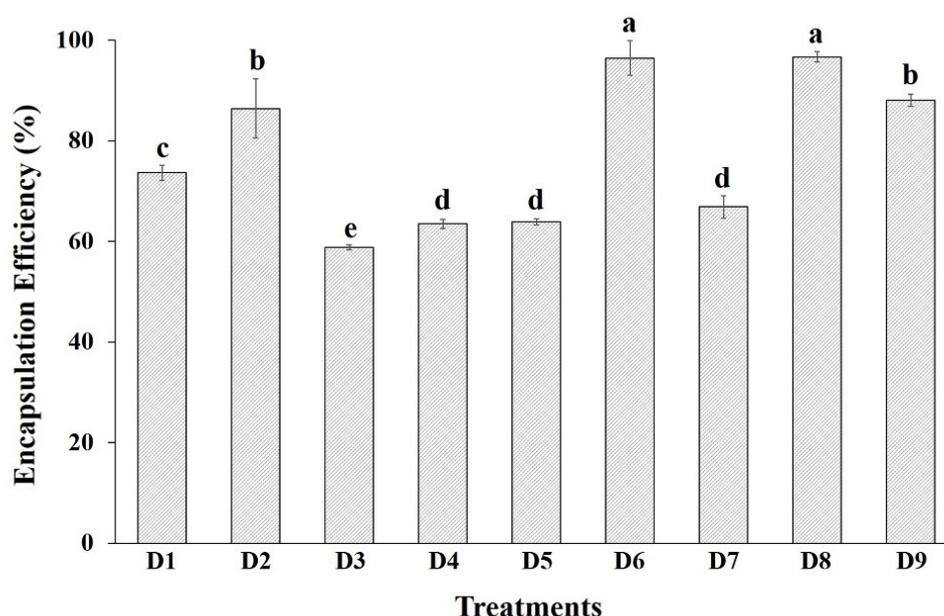


Figure 1 Encapsulation efficiency of wasabi favor beads for different bead formulations D1 = H0F1A10C1, D2 = H0F1A12C1, D3 = H0F1A14C1, D4 = H1F0A10C1, D5 = H1F0A12C1, D6 = H1F0A14C1, D7 = H1F1A10C1, D8 = H1F1A12C1, D9 = H1F1A14C1 (H, HI-CAP 100; F, Flavotec; A, Alginate; C, Chitosan). Error bars indicate standard errors. Letters with different superscripts indicate samples that are significantly ($p < 0.05$) from each other.

Effect of thermal processing on encapsulated wasabi flavor

To evaluate the effects of thermal processing on the model wasabi flavor beads stability, microwave heating and autoclaving were chosen. The impact of thermal processing on AITC retention of the encapsulated beads made from various concentrations of wall materials was expressed as EE values (Table 3) and the example of GC chromatogram of AITC in encapsulated beads after thermal processing are shown Figure 2. The results indicated that the type of thermal processing significantly influenced the retention of flavor content of encapsulated beads ($p < 0.05$). Interestingly, it is clear that the EE value of D6 formulation was highest (92.32 %) after the microwave heating for all treatments. The data is in agreement with the initial EE determined after the encapsulation process in Figure 1. This confirmed that wall material consisted of HI-CAP 100 and Flavotec at mass ratio of 1:0 (w/w) and alginate and chitosan at mass ratio of 14:1 (w/w) have efficiency to prevent flavor loss during microwave heating. The result was similar to the study of Najafi *et al.* [32] who reported that the use of HI-CAP 100 exhibited superior encapsulating properties and considerable barrier effect against diffusion and loss of core material under extreme environmental condition such as spray drying. Also, the increase of alginate level resulted in the entanglement of alginate and chitosan chains in the matrix of beads appears to be strong enough to protect the bead degradation by heating [33]. For autoclaving, encapsulated beads for all treatments resulted in a sharp decrease. The highest EE value was found in D6 treatment that exhibited 7.87 % retention. This decrease may be correlated to the high temperature and pressure leads to the bead shrinkage, resulting in the subsequent release of the flavor [34]. The data is in concordance with the report from Young *et al.* [35], that sterilization condition had significant effect on the physicochemical stability for encapsulated all-trans retinol and curcumin emulsions and had maximum effect on bioactive recovery. In spite of the EE values is decrease after autoclave, the AITC had strong pungency for recognized detection due to its low threshold. This result was supported by Eib *et al.* [36] who stated that AITC showed pungency perception and low sensory detection thresholds in the water-based matrix in the pre-study and main, the values were 0.135 and 0.121 mg/100 mL, and those in the oil-based matrix were 0.497 and 0.582 mg/100 mL, respectively. Thus, encapsulated wasabi flavor with HI-CAP 100 and Flavotec at mass ratio of 1:0 (w/w) and alginate and chitosan at mass ratio of 14:1 (w/w) (D6) is recommendable for food product under thermal processing.

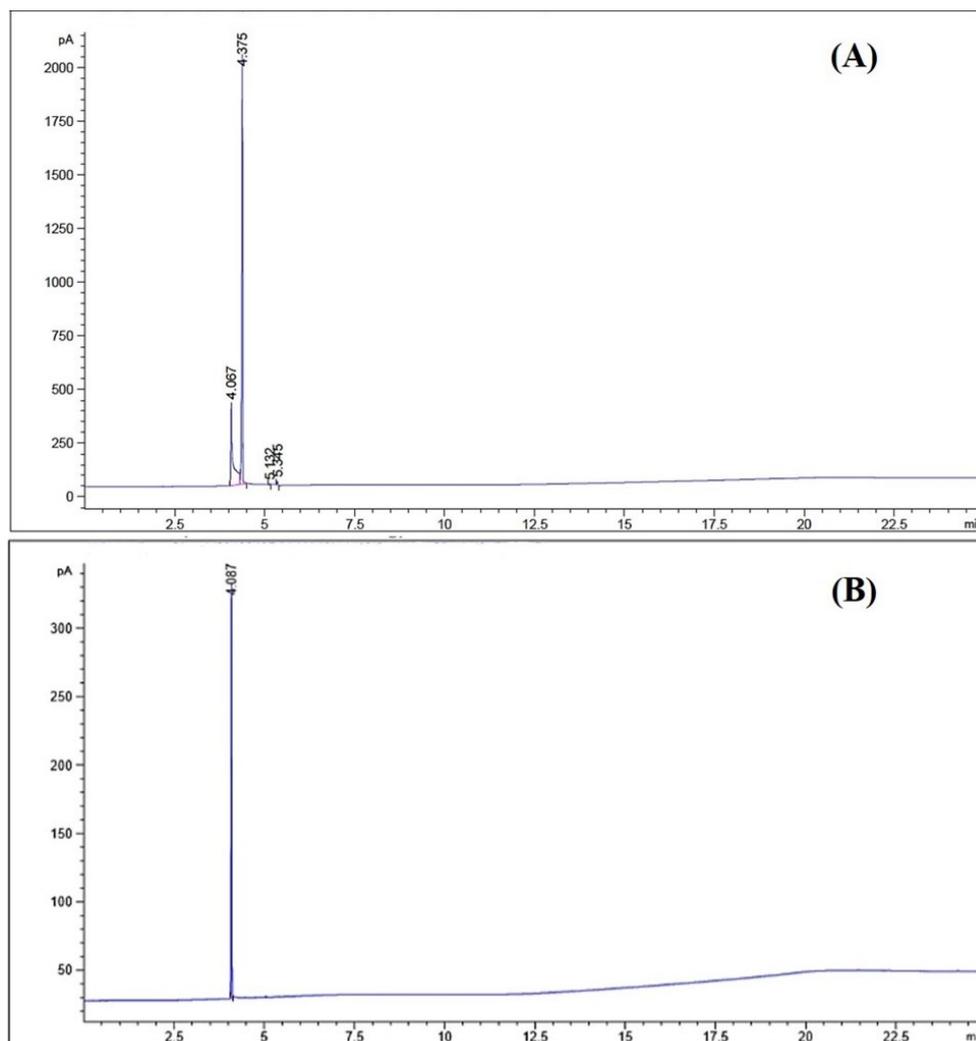


Figure 2 GC chromatograms of AITC in encapsulated beads before (A) and after thermal processing (B).

Table 2 Average diameter, weight and yield percentage of wasabi flavor beads made from wall materials with various concentration.

| Treatments | Diameter (mm) ^{ns} | Weight (mg) | Yield (%) |
|----------------|-----------------------------|----------------------------|---------------------------|
| D1 (H0F1A10C1) | 3.32 ± 0.02 | 43.83 ± 1.72 ^{cd} | 63.88 ± 1.34 ^d |
| D2 (H0F1A12C1) | 3.70 ± 0.02 | 52.77 ± 1.37 ^b | 76.60 ± 1.96 ^c |
| D3 (H0F1A14C1) | 3.43 ± 0.02 | 58.69 ± 1.48 ^a | 88.13 ± 0.49 ^a |
| D4 (H1F0A10C1) | 3.16 ± 0.02 | 46.35 ± 3.19 ^c | 64.08 ± 1.43 ^d |
| D5 (H1F0A12C1) | 3.35 ± 0.03 | 51.48 ± 1.27 ^b | 74.94 ± 1.59 ^c |
| D6 (H1F0A14C1) | 3.34 ± 0.03 | 57.29 ± 1.87 ^a | 84.35 ± 1.04 ^b |
| D7 (H1F1A10C1) | 3.51 ± 0.02 | 41.33 ± 1.05 ^d | 61.70 ± 1.64 ^d |
| D8 (H1F1A12C1) | 3.37 ± 0.02 | 53.63 ± 0.74 ^b | 75.31 ± 1.18 ^c |
| D9 (H1F1A14C1) | 3.43 ± 0.02 | 59.99 ± 1.19 ^a | 89.91 ± 1.10 ^a |

Abbreviations; H, HI-CAPTM 100; F, Flavotec D30; A, Alginate; C, Chitosan
ns = not significant.

^{a-d} Mean values in the same column followed by the different letters are significantly different ($p < 0.05$).

Table 3 Retention of AITC in encapsulated beads made from wall materials with various concentration after thermal processing.

| Treatments | Encapsulation Efficiency (%) | |
|----------------|------------------------------|---------------------------|
| | Microwave Heating | Autoclave |
| D1 (H0F1A10C1) | 3.09 ± 0.22 ^h | 1.64 ± 0.04 ^e |
| D2 (H0F1A12C1) | 29.27 ± 0.77 ^f | 4.03 ± 0.17 ^d |
| D3 (H0F1A14C1) | 33.36 ± 0.75 ^e | 7.09 ± 1.53 ^a |
| D4 (H1F0A10C1) | 22.81 ± 1.39 ^g | 3.72 ± 0.59 ^d |
| D5 (H1F0A12C1) | 61.45 ± 1.19 ^b | 5.87 ± 0.36 ^b |
| D6 (H1F0A14C1) | 92.32 ± 4.94 ^a | 7.87 ± 0.19 ^a |
| D7 (H1F1A10C1) | 25.17 ± 2.67 ^g | 4.68 ± 0.57 ^{cd} |
| D8 (H1F1A12C1) | 42.93 ± 1.55 ^d | 4.66 ± 0.41 ^{cd} |
| D9 (H1F1A14C1) | 48.09 ± 0.77 ^c | 5.66 ± 0.47 ^{bc} |

Abbreviations; H, HI-CAP™ 100; F, Flavotec D30; A, Alginate; C, Chitosan

^{a-h} Mean values in the same column followed by the different letters are significantly different ($p < 0.05$).

Conclusions

This study was performed to investigate the resistance high temperature of encapsulated wasabi flavor in order to extend stability of ingredient in food processing. The beads with 10% (w/w) wasabi flavor in a blend of HI-CAP 100 and Flavotec at mass ratio of 1:0 (w/w) and alginate and chitosan at mass ratio of 14:1 (w/w) used as wall materials provides sufficient protection and efficiency of AITC under microwave heating and autoclaving. Our findings demonstrated that the encapsulated wasabi flavor generated under the studied conditions had capability to protect degradation of AITC under the high temperature condition. These results constitute a theoretical foundation for the application of encapsulated wasabi flavor as additive in the food industry.

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