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Quality changes and FTIR characteristics of the house cricket (*Acheta domesticus*) during frozen storage

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Abstract

This research aimed to evaluate the quality changes during frozen storage (0-4 months) of antioxidant (0.02% Butylated hydroxyanisole (BHA): Butylated hydroxytoluene (BHT)) glazed cricket (AGC). The protein and lipid changes in frozen cricket were determined compared to control (without antioxidant). The results showed that lipid and protein oxidations as evidenced by Thiobarbituric acid reactive substances (TBARS) and protein carbonyl values, respectively, of the control were greater than the AGC up to 2 months of storage ($p \leq 0.05$) and then decreased to the comparative levels. The protein solubility of the AGC sample revealed lower values than those which were concomitant with a differential scanning calorimetry (DSC) analysis exhibiting higher thermal stability compared to the control sample. This probably resulted from phenolic antioxidant-protein interactions. The concentrated protein powder from the AGC had a lower rate of lipid oxidation than that from control as proved by the specific Fourier Transform Infrared Spectroscopy (FTIR) bands (i.e. the ratio of absorbances at 3008 cm^{-1} to 2955 cm^{-1} and absorbances between 3600-3100 cm^{-1}). These findings suggest that glazing crickets with antioxidants can minimize protein and lipid deviations during frozen storage.

Keywords: House cricket, frozen storage, antioxidant glazing, DSC, FTIR

1. Introduction

The world's expanding population, the recent experience of a pandemic disease, and the ongoing impact of war on food security and demand for food from sustainable sources has created serious problems. In addition, the need for new, unconventional sources of food is being driven by global environmental changes. Insects have been identified as alternative protein sources that might fulfill future protein demands [1]. Furthermore, the production of insects has various benefits over other protein sources, including lower greenhouse gas emissions, diminished water and land utilization, and greater protein conversion efficiency. Insect proteins have recently attracted attention from the food industry since they are a good resource of protein (around 40%-70% on a dry weight basis), minerals, vitamins, and polyunsaturated to saturated fatty acid ratios [2, 3]. Thailand is the most important insect exporter, especially with regard to crickets of which there is a total production capacity of more than 7,000 tons [4]. The house cricket (*Acheta Domestica*) has approximately 59.7% protein, 23.8% fat, 4.6% ash, and 11.9% carbohydrate [5]. Since cricket production is expanding faster than demand, crickets must be preserved mostly by freezing while waiting for sale. However, the frozen cricket, such as *Acheta Domestica*, has a high fat content (23-29%) [6], with monounsaturated fatty acid and polyunsaturated fatty acid content of 24.5% and 37.0%, respectively [5]. Lipid oxidation, which can cause off-odor and off-flavor, is a major issue [7]. Solute concentration is increased during freezing, which might accelerate lipid oxidation. During freezing, free radicals produced during lipid oxidation can cause protein oxidation and denaturation.

Proteins are immense and complicated molecules, and oxidation can alter the protein side chains and backbone, including peptide bond cleavage and protein crosslinking, which cause changes in protein hydrophobicity, conformation, polymerization and precipitation [8, 9] resulting in changes in solubility, color, taste, and other characteristics of the final products.

Food preparation before freezing, such as blanching and dipping in an antioxidant solution, can prevent some undesirable changes in food. Mancini et al. [10] reported that blanching *Tenebrio molitor* at 60°C for 5 minutes can inhibit microbial growth. Furthermore, blanching can prevent browning by inactivating the enzyme that causes the browning reaction. Shi et al. [11] studied the effects of shrimp that were dipped in rosemary extract (0.2%), a natural antioxidant, and then frozen at -40°C for 26 weeks compared to shrimp that were dipped in water and shrimp that were not dipped. According to the study [11], shrimp dipped in rosemary extract can prevent oxidation better than shrimp dipped in water. The purpose of this study was to investigate the chemical and physicochemical changes such as protein and lipid oxidation, thermodynamic properties, as well as molecular structural alterations using the Fourier-transform infrared (FTIR) technique for frozen cricket (*Acheta domesticus*) with and without antioxidants (butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) glazing during frozen storage.

2. Materials and methods

2.1 Materials

Adult crickets (45-days-old) were purchased from a local farm in Khon Kaen province, Thailand. Samples were packed in a plastic bag and then transferred to Khon Kaen University within 1 h. The crickets were washed and boiled (95-98°C) in water for 15 min and cooled by air. They were soaked in an antioxidant solution (0.02% BHA:BHT (1:1)) for 30 min (4-5°C) [7]. Then, the soaked crickets were removed and allowed to drain in a perforated basket for 30 min. Antioxidant-soaked crickets or control (without antioxidants) were vacuum packed in an aluminum foil bag and were frozen in an air-blast freezer (Air Blast freezer, Rivacold Ice 13-10, Tewkesbury, Gloucestershire, UK) at -35°C for 2-3 h (until the core temperature reached -18°C) thus obtaining frozen antioxidant glazed crickets (AGC). The frozen samples were stored at -20±2°C in a freezer (SF-C995, Sanyo, Bangkok, Thailand). Samples were taken to be analyzed at 0, 1, 2, 3 and 4 months.

Before the analyses, samples were thawed and washed with distilled water then allowed to drain. The samples were ground by a meat grinder (BIRO 8-22 E97, The BIRO MFG. Co., USA) through a hole-plate (5 mm diameter). All samples were ground twice.

2.2 Lipid oxidation

A thiobarbituric acid reactive substances (TBARS) assay was performed in the same way as [12] with a slight modification. In brief, minced cricket was mixed with distilled water at 10,000 rpm for 1 min (ACE, Nihonseiki Kaisha Co., Japan homogenizer). The homogenate was then poured into a distillation tube and treated with 5N HCl. After distillation (Distillation Unit, Kjeltec^{TM}8100, Foss, Sweden), the distillate was collected in a test tube and then 2-thiobarbituric acid in 90% acetic acid was added. The distillate was heated for 30 min and then cooled. The absorbance was measured at 538 nm using a UV-visible spectrophotometer (Lambda 25, Perkin Elmer, Shelton, CT) and 1,1,3,3-tetra-ethoxypropane (TEP, Sigma) as the standard (mg/kg sample).

2.3 Protein oxidation

The protein oxidation (protein carbonyl) of both samples (AGC and control) were measured as described by [13] with a slight modification using the 2,4-dinitrophenylhydrazine (DNPH) derivatization. A three-gram sample of minced cricket was mixed with 15 mL of 20 mM phosphate buffer (pH 6.5) containing 0.6 M NaCl using a homogenizer (ACE, Nihonseiki Kaisha Co., Japan homogenizer) at 10,000 rpm for 1 min. The homogenates were divided into two equal aliquots of 0.2 mL, then were mixed with 1 mL of 10%(v/v) trichloroacetic acid (TCA) to precipitate protein and incubated at 4-5°C for 15 min. Following centrifugation (Hitachi high-speed refrigerated centrifuge CR22N, Eppendorf Himac Technologies Co., Ltd., Japan) (6,000 xg, 10 min, 4°C), 1 mL of 10 mM DNPH was added to the pellets. A sample blank was prepared with 1 mL of 2 N HCl. The mixtures were kept in the dark for 1 h before adding 0.5 mL of cooled 20% (v/v) TCA and then centrifuged at 6,000 xg for 10 min (4°C). The pellets were washed twice with ethanol/ethyl acetate (1:1 v/v) and dissolved with 1.5 mL of 6 M guanidine-HCl in 20 mM phosphate buffer (pH 6.5). After incubation in the dark (30 min), the mixtures were centrifuged at 6,000 xg for 10 min

(4°C) and the absorbances of the supernatant were measured at 280 and 370 nm. The relative protein concentration was calculated using Bovine serum albumin (BSA) as standard. The carbonyl content was calculated using the following equation and the results are presented as nM of carbonyl per mg of protein.

$$\frac{C_{hydrazone}}{C_{protein}} = \frac{A_{370}}{\varepsilon_{hydrazone,370} \text{ x } (A_{280}-A_{370} \text{ x } 1)} \times 10^6$$

When $\varepsilon_{hydrazone,370} = 22{,}000\ M^{-1}cm^{-1}$

$C_{hydrazone}$ and $C_{protein}$ are the amounts of protein carbonyl and protein, respectively.

2.4 Protein Solubility

Protein solubility was assayed using the same method as [14] with slight adjustments. Briefly, 0.05 g of each minced sample was dissolved in 8 mL of distilled water. pH of the mixture was altered to 3-8 using 0.2 and 1 M NaOH and 0.2 and 1 M HCl. The total protein of the minced sample was dissolved in 0.5M NaOH. After stirring for 30 min, distilled water was added to a final volume of 10 mL. The mixture was then centrifuged at 6,000xg for 10 min (25°C). The protein content in the supernatant was determined using the Lowry [15] with BSA as a standard. The percentage of protein solubility was calculated as follows:

$$\text{Protein solubility (\%)} = \frac{\text{Protein content of supernatant}}{\text{Total protein content of sample}} \times 100$$

2.5 Differential scanning calorimetry (DSC)

Thermal properties of AGC and control were evaluated using a DSC 4000 furnace (PerkinElmer, Waltham, MA, USA). Before use, the DSC was calibrated by the indium standard. The minced samples were passed through a sieve (with square-shaped 16 mesh (1.18 mm). Fifty milligrams of the sample were put a DSC sample pan and the temperature range was set from 25°C to 190°C, with a heating rate of 5°C/min. The DSC data including onset, peak, end, and delta H were evaluated using Pyris 6 data analysis software (Perkin Elmer).

2.6 Fourier-transform infrared (FTIR) spectrum analysis

A cricket protein concentrate powder (CPC) was prepared for FTIR analysis. The minced cricket was boiled in alkaline water and yielded an alkaline treated cricket suspension. Foam-mat drying of the suspension was performed to achieve CPC [16]. The CPC was placed onto the ATR crystal cell of the ATR-FTIR (attenuated total reflectance-FTIR spectrometer) equipped with a deuterated glycine sulfate detector (DTGS) (Bruker, TENSOR 27 System, Germany). The obtained FTIR spectra were conducted at a 4 cm^{-1} resolution with 16 scans. The spectral data were collected and preprocessed using the OPUS 7.0 software program (Bruker Co.) and then they were imaged by MagicPlot student 2.5.1.

2.7 Statistical analysis

All experiments were run in duplicate. The results were presented as mean ± standard deviation. Analysis of variance (ANOVA) was applied with IBM SPSS statistics version 19 (IBM Corp., Armonk, NY, USA). The significant differences of the means among treatments were determined using Duncan's new multiple range test (DMRT) and they were defined at $P<0.05$.

3. Results and discussion

3.1 Lipid oxidation

The lipid oxidation of both control and AGC samples during frozen storage were evaluated using the thiobarbituric acid-reactive substances (TBARS) technique. The results show that there was an interaction ($P\leq0.05$) between antioxidant glazing and storage time (data not shown). The highest TBARS values of 0.186 and 0.200 mg Malondialdehyde (MDA)/kg

crickets for AGC and control at 0 month (crickets before freezing), respectively, were revealed (Figure 1A). This probably resulted from the boiling treatment which promoted lipid oxidation. According to Weber et al. [17], the increased TBARS values after boiling could have been due to the high temperature. The oxidation threshold for fishmeal suggested that TBARS values lower than 1.5 mg/Malondialdehyde (MDA)/kg fall in the category of "not rancid" [18] thus during 4 months frozen storage of both AGC and control, very low secondary products of lipid oxidation were noted. The TBARS values ($P \leq 0.05$) were lower for AGC than those of control observed during 1 to 3 months indicating that glazing with antioxidants can delay the lipid oxidation of frozen crickets. The TBARS of control dramatically decreased to comparable level to that of AGC's TBARS after 2 months. Similar findings were reported by Wachirattanapongmetee et al. [19]. They noted an increase in TBARS after 5 freeze-thaw (F-T) cycles of fish fillet dipped in protein hydrolysate from tilapia byproducts which possessed an antioxidant function. Then a decline of TBARS after 10 FT cycles was seen since malonaldehyde, a secondary product of lipid peroxidation can be oxidized to other compounds which cannot be determined by the TBARS method.

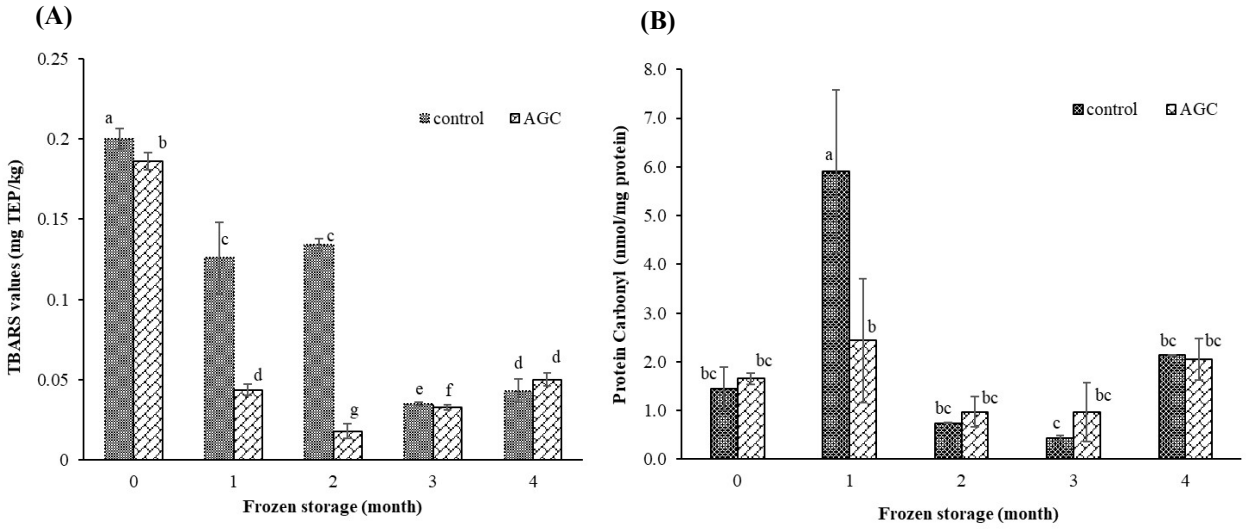

Figure 1 2-Thiobarbituric acid-reactive substances (TBARS) values of antioxidant glazed crickets (AGC) and control during frozen storage (0-4 months) (A). Protein oxidation of antioxidant glazed crickets (AGC) and control during frozen storage (0-4 months) (B). All data are the mean ± standard deviation (SD). Values with different subscripts in each picture (A,B) are significantly different ($p < 0.05$).

3.2 Protein oxidation

Protein carbonyl content of crickets stored under different treatments and times was investigated and the findings are shown in Figure 1B. Antioxidant treatment and storage time had an interaction effect on the carbonyl content ($P \leq 0.05$) (statistical data not shown). Non-oxidized muscle tissue has a carbonyl level of 1 nmol/mg protein, but oxidized muscle tissue has a carbonyl content ranging between 2 and 14 nmol/mg protein, which is affected by the rate of oxidation, muscle type, and the source of oxidation [20, 21]. In this study, at 0-month storage, control and AGC showed carbonyl content of 1.44 and 1.65 nmol/mg protein, respectively. After 1 month, the carbonyl content of control sharply increased to the highest level (5.9 nmol/mg protein) and then declined to a comparable level to those of AGC after 2 to 4 months' storage while the protein carbonyl content of AGC was ranged between 0.43 and 2.44 nmol/mg protein. This signifies that antioxidants might delay the protein oxidation of crickets during frozen storage. The variations in carbonyl content during frozen storage might be due to the interaction between carbonyl derivatives and other cell components [22]. Estévez et al. [23] found a reduction of protein carbonyls in various sources of proteins during storage at 37°C, 14 days after they had further interacted with other compounds such as cross-links and Schiff bases reactions between protein semialdehydes and functional groups of amino acids.

3.3 Protein solubility

Solubility is considered as a crucial functional characteristic when developing a novel protein component [24]. Environmental parameters, specifically pH, have a significant impact on protein solubility. The pH models at 4 and 7

were applied in this study since most foods fall into the categories of acidic and neutral conditions. It can be observed that both pH and storage time significantly affected the protein solubility of frozen crickets (Figure 2). In general, at neutral pH, proteins in frozen crickets were more soluble than in acidic pH, which could be due to the isoelectric pH of the major proteins in house cricket flour [25]. Similarly, the lowest protein solubility of house cricket flour after refrigerator storage was observed to be in acidic pH, while protein solubility increased considerably in alkaline pH (pH 8-10) [25]. At the initial storage stage, the greater protein solubility of control compared to AGC was noted for both pHs. The reduced protein solubility of AGC in month 0 could have been due to the reaction between BHA and BHT (phenolic compounds) and proteins forming complexes via covalent linkages and/or non-covalent interactions resulting in lowering protein solubility [26]. The lowest protein solubility at both pHs (0.95-2.05%) was observed in both control and the AGC samples which were stored for 1 month. Janssen et al. [27] revealed that blanching insects (*Tenebrio molitor, Alphitobius diaperinus* and *Hermetia Illucens*) decreased protein solubility and lowered the hydrophobic amino acids in the soluble protein extract compared to untreated samples. This was likely caused by protein aggregation due to more hydrophobic amino acids contained in the heat-treated insects as well as the phenolic-protein complexes. Interestingly, the protein carbonyl contents of both samples at 1-month storage demonstrated high values (Figure 1B), especially for control. This might have caused low protein solubility resulting from protein crosslinking [28]. Afterwards, the solubility of both treatments considerably increased and then changed slightly during the period of 2 to 4 months' storage. This could possibly have resulted from the autolysis of the extended time that the frozen crickets were in storage.

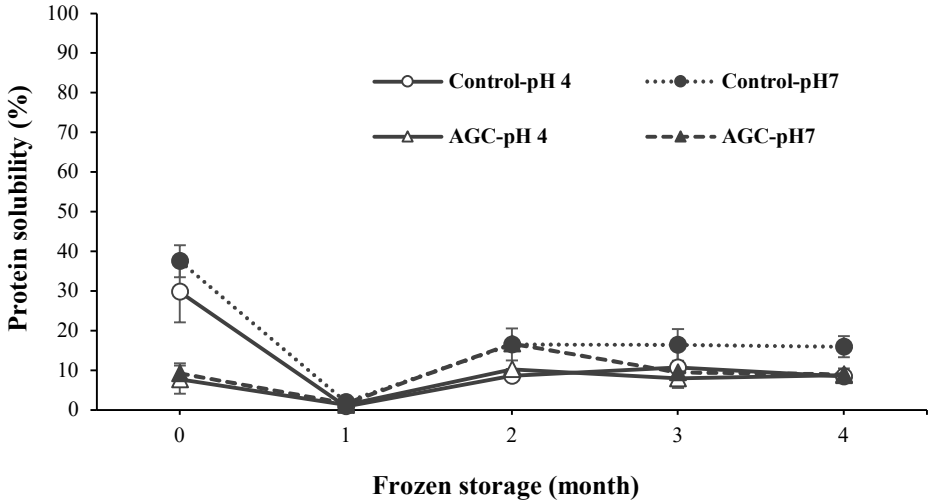

Figure 2 Protein solubility (pH 4 and pH 7) of antioxidant glazed crickets (AGC) and control during frozen storage (0-4 months).

3.4 Differential Scanning Calorimetry (DSC)

The thermal characteristics of a substance, i.e. the heat-induced denaturation of proteins, can be revealed by a DSC analysis [29]. The heat flux with a single endothermic peak of the fresh crickets, control, and AGC samples through frozen storage were not significantly different with the enthalpy of 963.3-1205.5 J/g and a peak temperature (T_p) which ranged from 105.9 to 117.2°C (Table 1). Ruggeri et al. [30] determined the thermal properties of spray dried house crickets and reported four endothermic peaks at around 84.5°C, 196.3°C, 339.5°C, and 563.3°C where the latter two peaks showed significant enthalpy changes. The difference between these might be the results of the different thermal treatments experienced by the samples. These samples [30] were pasteurized, minced, and milled before spray drying with an inlet temperature of 200°C whereas our samples were boiled for 15 min before soaking and freezing. The peak temperature (T_p) of the chitin extracted from our boiled sample (without freezing) was determined to compare with the ground whole crickets and it was found that the T_p of the chitin extract revealed four endothermic peaks (145-345°C) with the main peak at around 145°C (data not shown). Torruco-Uco et al. [31] studied the thermal characteristics of grasshopper flour (dried at 55°C, 24 h and ground), obtaining a single peak at 131.01-163.07°C with an enthalpy of 5059 J/g. They speculated that this peak possibly related to the protein-lipid complex forming in grasshopper flour while drying.

Table 1 DSC parameters (peak temperature; T_p and enthalpy of denaturation; ΔH) of antioxidant glazed crickets (AGC) and control (crickets without antioxidants) during frozen storage (0-4 months) compared to fresh crickets (FC).

Samples	Storage time (month)	T_p (°C)	ΔH (J/g)
FC	-	105.9 ± 10.4	1008.5 ± 70.8
AGC	0	108.1 ± 1.9	1144.8 ± 48.9
Control		117.2 ± 3.2	963.3 ± 68.5
AGC	1	114.9 ± 3.9	1061.7 ± 95.2
Control		108.9 ± 1.1	1001.5 ± 46.2
AGC	2	115.6 ± 1.6	1205.5 ± 37.1
Control		106.9 ± 0.9	1018.9 ± 66.6
AGC	3	107.5 ± 1.3	1008.0 ± 77.9
Control		107.4 ± 0.6	971.9 ± 80.3
AGC	4	106.3 ± 0.5	985.1 ± 28.0
Control		107.0 ± 1.0	994.6 ± 40.3

During frozen storage, at 0 month the T_p of control was higher than fresh crickets and AGC which could be attributed to native protein changes caused by heat (boiling for 15 min) induced protein aggregation. Although the effect of antioxidants in AGC can delay the reaction, its T_p increased considerably at 1- and 2-months' frozen storage. The binding of antioxidants which contain phenolic structures and proteins results in complexations which may cause an increase in T_p. Several studies have shown that phenolic compounds can form complexes with proteins resulting in a decrease in protein solubility with greater thermal stability [26]. The T_p of control and AGC was then reduced after 1- and 3-months' storage, respectively.

3.5 Fourier transform infrared (FTIR)

The FTIR spectroscopy technique can be used to evaluate the molecular diversity of the samples comparatively based on a knowledge of the peak origins, such as proteins, nucleic acids, lipids, and carbohydrates [32]. Figure 3 shows the FTIR spectrum of the cricket protein concentrate powders derived from AGC and control which were kept frozen for 0, 2, and 4 months. Figure 4 represents the changes in the absorbance and absorbance ratio of the specific wave numbers of control and AGC during frozen storage. Our results show that the ratio of A_{3008}/A_{2955} (Figure 4A) and band intensity at 3600-3100 cm^{-1} (Figure 4B), which indicate lipid desaturation (adding a double bond to a fatty acid chain) and products of lipid peroxidation i.e. hydroperoxide, alcohols and other compounds derived from hydroperoxides, respectively, demonstrated a decreased trend when the storage time was extended and had positive correlations (R = 0.9266 and 0.8449, respectively) with the TBARS (Figure 1A). The C-H stretching vibration of the cis double bond (C=C) was assigned to the band at 3012 cm^{-1}. Daoud et al. [33] reported a reduced intensity of a weak band at 3012 cm^{-1} of the oil in water emulsion after 15 days' storage. Lipid oxidation in red carp caused a reduction in the concentration of unsaturated acyl chains during frozen storage [34]. The ratios between the FTIR intensities 3012 cm^{-1}/2960 cm^{-1} were allied with lipid desaturation. An absorbance ratio of A_{3013} /A_{2960} was the lowest in H_2O_2-treated cells from rat enterocytes compared with H_2O_2-treated cells plus different polyphenols [35]. The hydroxyl region between 3600 and 3100 cm^{-1} responded to the stretching vibrations of OH from water, hydroperoxides (primary products of lipid oxidation), and their deteriorated products (aldehydes, alcohols, and ketones). Degraded compounds produced as oxidation progresses caused the shifting and the broadening of this band [36]. Several changes in lipid structures have been related to various FTIR frequencies such as the bands near 3434, 3440 or 3458 cm^{-1}, and 3530 cm^{-1} which are associated with hydroperoxide groups produced in the lipid oxidation of various edible oils and alcohols as well as other compounds derived from hydroperoxides, respectively [36]. Our study revealed an intensity in the reduction of the band between 3600 and 3100 cm^{-1} of both control and the AGC samples (Figure 4B). A concomitant descendant of TBARS and absorbance at 3600-3100 cm^{-1} could have been due to the alteration of the secondary product of lipid peroxidation to other compounds.

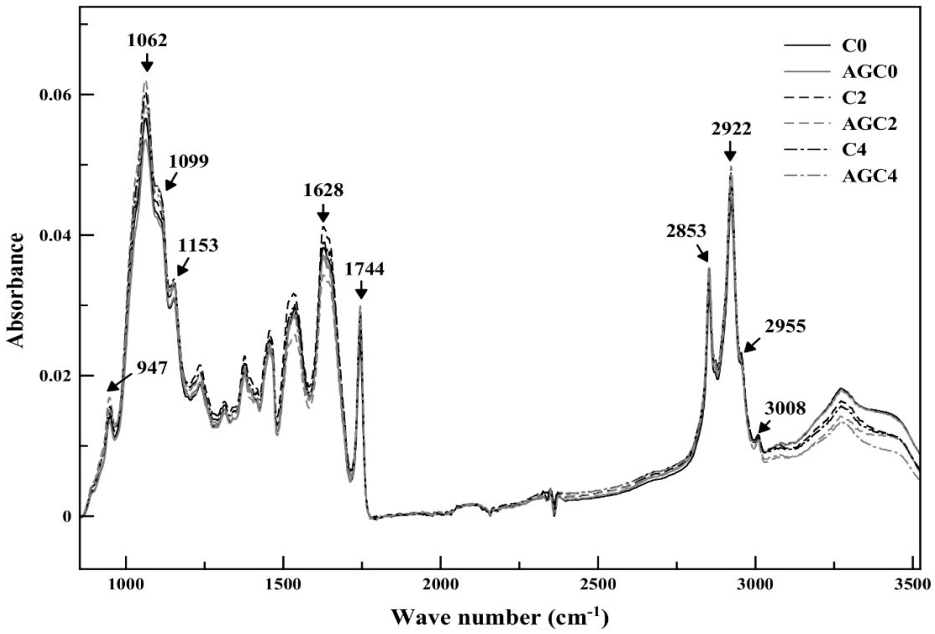

Figure 3 FTIR spectra of antioxidant glazed crickets (AGC) and control (C) during 0, 2, and 4 months frozen storage.

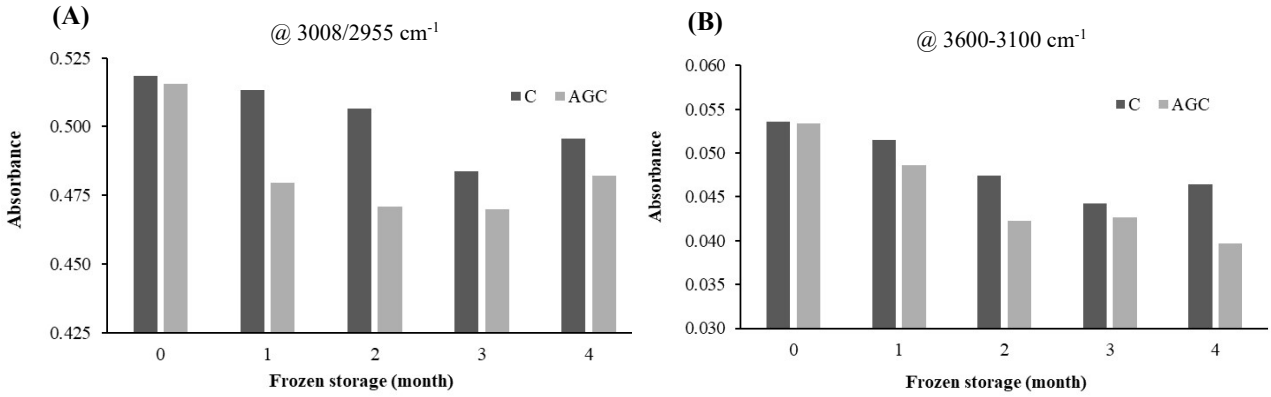

Figure 4 Changes in the absorbances of A_{3008}/A_{2955} (A), and $A_{3600\text{-}3100}$ (B) of antioxidant glazed crickets (AGC) and control (C) during 4 months' frozen storage.

4. Conclusion

Antioxidants (BHA and BHT) can slow down lipid and protein oxidation in crickets during frozen storage. However, antioxidant and heat treatments possibly caused phenolic antioxidant-protein interactions as evidenced by lower protein solubility and greater thermal stability of antioxidant glazed crickets compared to control. The changes in the intensity and frequency of different bands and/or ratios between the absorbances of some bands could be used practically for monitoring the lipid oxidation in crickets during storage. Further molecular studies involving protein aggregation and phenolic antioxidant-protein interaction affecting cricket qualities are essential.

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