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Research Article

The effect of storage temperature on bioactive compounds, the oxidation of lipid and free radical scavenging capacity to peanut butter product

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Abstract

This study aims to evaluate the effect of storage temperature on the content of bioactive compounds (resveratrol, biochanin A, and genistein), the free radical scavenging capacity, and lipid oxidation of peanut butter product through the peroxide value (PV), total fat (TF), and free fatty acid (FFA) content. Peanut butter supplemented with 2% olive oil and 4% honey was stored at two temperature levels: cool temperature (8 - 10°C) and room temperature (28 - 30°C) for 8 months. Research results showed that peanut butter stored at the cool temperature (8 - 10°C) is more suitable than at the room temperature (28 - 30°C). Under these storage conditions, the contents of resveratrol, biochanin A, and genistein were 2.94 mg/kg, 1.83 mg/kg, and 2.83 mg/kg, respectively. The antioxidant activity, assessed through DPPH evaluation, reached 68.12%. Additionally, the TF (47.05%), FFA (0.66%), and PV (0.4844 meq/kg) were also recorded.

Keywords: *bioactive compounds, free radical scavenging, lipid oxidation, peanut butter, storage temperature*

1. INTRODUCTION

Peanuts (*Arachis hypogaea* L.) are a nutrient-rich food source whose components included high content of protein, fat, as well as essential micronutrients and bioactive compounds [1-2]. Notably, the content of monounsaturated fatty acids (MUFAs) in peanuts ranges from 49-57% [1]. In addition to their high MUFA content, peanuts also provide valuable vitamins and minerals, such as vitamin E and magnesium [3]. Furthermore, peanuts contain various biologically active compounds, including resveratrol (5.6 - 29.6 µg/kg), biochanin A (0.133 - 0.964 mg/100 g), genistein (0.3 mg/100 g). These compounds have demonstrated the antioxidant effect, which induce the protection of cardiovascular and nervous system, and improvement of diabetes and obesity [4-7].

Processing peanuts in avocado form are considered to have little impact on nutrient content and enhancing peristalsis and promoting the efficient absorption of proteins and MUFAs while increased peristalsis, and easy absorption of proteins and MUFAs contained in the product [2]. Peanut butter is widely known as a nutritious and energy-rich product, thus biologically active compounds and the antioxidant properties and susceptibility to lipid oxidation during storage at varying temperatures of peanuts did not well awareness. Consequently, the aim of this study was figured out the influence of temperature conditions on quality of peanut butter.

2. MATERIALS AND METHODS

2.1. Materials

MD7 peanuts were used as the raw material in this study. After harvesting, peanuts was dry until the humidity drop to 7- 8%. During the study period, dried peanuts was stored in vacuum-sealed PA bags and frozen for a maximum period of two months, following the standards outlined in TCVN 2383:2008. All of

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peanuts in this study was collected at Bao Tram Cooperative (Soc Moi hamlet, Long Son commune, Cau Ngang district, Tra Vinh province).

Olive oil (Pomace, Spain): acidity: 3%, peroxide value: 5 meq/kg, stearic acid 0.5 - 5.0%, linoleic acid 3.5 - 21.0%. Honey (Phuong Nam, Vietnam): including water <20%; glucose and fructose > 80%; sucrose < 5%).

Additives added to peanut butter include: antioxidant BHT was purchased from Dengfeng, China), Lecithin was used as emulsifier from China.

2.2. Research methods

2.2.1. Sample preparation and experiment design

MD7 peanuts (dried seeds) after purchasing from the cooperative are peeled and preliminarily cleaned. Then, roast at a temperature of 200°C for 20 minutes to create a characteristic aroma and beautiful color for the product. Next, the peanuts were put at room temperature to cool down, and the silk shells and burnt seed are removed. A total of 300 g of roasted peanuts was weighed and grind twice (30 seconds per grind) to form a paste. Other ingredients was mixed based on the optimal formulation, that was synthesized from previous study, including 2% olive oil, 4% honey, 0.2% lecithin (w/w), and 100 mg/kg fat of BHT. The peanut butter mixture is stored in a transparent PET container (100 g/box).

Process diagram: dried peanuts → preliminary cleaning (impurity removal) → roasting → fine grinding → ingredient mixing → packaging (100g) and storage.

The experiment was designed using randomized model with 2 factors including temperature and storage duration, and 3 replications. In detail, the storage temperature was set at 8°C for cold temperature and 28°C for room temperature. Total samples: 2 x 3 x 8 = 48 samples.

2.2.2. Analysis methods

- Determination of biologically active compounds:

Resveratrol, biochanin A and genistein were analyzed by HPLC using Ecosil-C18 (250 mm × 4.6 mm, 5 µm) column as station phase. Mobile phase was set at isocratic mode, and TF was 1.0 mL/min using deionized water (DI water): Methanol: Acetonitrile: Phosphoric acid (H_3PO_4) (60:30:38:1, v/v/v/v). The temperature of column chamber was maintained at 40°C during analysis time (25 minutes) with an injection volume of 10 µL. Detection was performed using a UV detector set at 262 nm for genistein and biochanin A 306 nm for resveratrol [9].

- Evaluation of DPPH free radicals scavenging ability [10]:

A 2.0 g sample of peanut butter was weighed and mixed with absolute methanol at a 1:6 (w/v) ratio, then frozen for approximately 12 hours. The sample was subsequently subjected to cold ultrasonic extraction for 10 minutes and filtered to obtain a clear extract. A 3 mL aliquot of the extract was combined with 1 mL of 0.1 mM DPPH solution, vortexed, and incubated in the dark at room temperature for 30 minutes. Optical absorption was measured at 517 nm. A blank sample was prepared in the same manner, substituting methanol for the sample extract. The percentage reduction of DPPH free radicals was then calculated using the standard formula:

$$\text{DPPH (\%)} = [(Abs_{blank} - Abs_{sample})/Abs_{blank}] \times 100$$

In which: Abs_{blank} is the absorption of DPPH solution mixed in methanol, Abs_{sample} is the absorption of test samples + DPPH.

- Determination of total lipid content (TF) was conducted following the AOAC method (2000 945.16) [11].

- Peroxide index (PV) was conducted according to TCVN 6121:2018 [12].

- Free fatty acids (FFA) content was implemented according to TCVN 6127:2010 [13].

2.2.3. Data processing methods

The experiment was conducted with three replicates, and the results are presented as the mean of three trials ± standard deviation. Statistical analysis, including variance analysis and LSD testing at a 5% significance level, was performed using the Statgraphics Centurion 16.1 software. The data shown represent the average values obtained from the experiments.

3. RESULTS AND DISCUSSION

3.1. Modification of bioactive substances during peanut butter storage

3.1.1. Variation in resveratrol content

Figure 1 illustrates the resveratrol content in peanut butter samples stored at two different temperatures over an eight-month period. The results showed that the resveratrol content of peanut butter samples significantly decreased during storage ($p<0.05$). Peanut butter products before storage contain 6.26 mg/kg resveratrol. After 8 months of storage, the remaining resveratrol content in peanut butter samples was 2.94 mg/kg at cold temperature and 2.13 mg/kg at room temperature. The percentage loss of resveratrol was 53.04% in cold conditions and 65.97% at room temperature. From the 2nd month, the rate of resveratrol degradation varied significantly between the two temperature conditions. Specifically, in March, April, and May, the monthly decline at room temperature ranged from 7.34% to 10.22%, whereas cold storage preserved resveratrol better, with a reduction rate of 5.27% to 7.82% per month. Particularly in the 8th month, the rate of decline in resveratrol content began to slow down with 6.71% per month at cold temperature and 10.70% per month at room temperature. Thus, the rate of loss of resveratrol content in peanut butter in room conditions is strong compared to cold conditions. This result is consistent with the publication of Zupančič *et al*. (2015) [14], which demonstrated that cold temperatures limited exposure to oxygen and light are suitable conditions for trans-resveratrol storage.

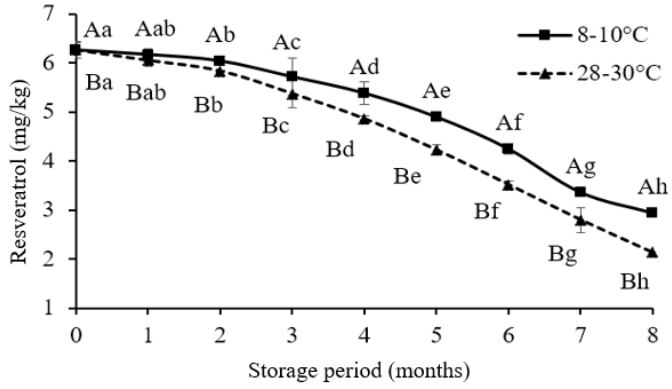


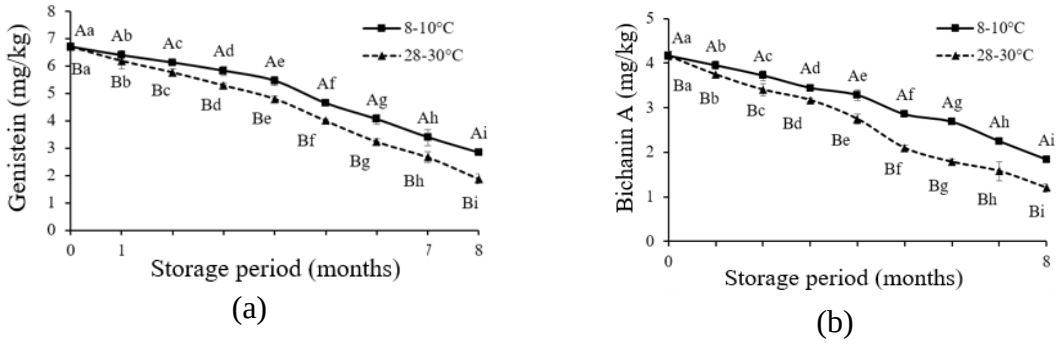
Figure 1. *Graph showing the effect of storage time and temperature on the resveratrol content of peanut butter. Notes: The letters A, B, C, D, ... (Storage temperature) and a, b, c, d, ... (Storage time), different represent statistically significant differences (P < 0.05) and vice versa.*

3.1.2. Variation in genistein and biochanin A content

Peanut butter's quality can be reduced by humidity and temperature which degrade Genistein, a polyphenol of interest. Storage length and temperature have a prominent correlation with the levels of Genistein and Biochanin A in peanut butter like the images shown in **Figure 2**. Genistein content, similarly to resveratrol, gradually decreased over time (**Figure 2a**), but resveratrol sample stored in cold conditions decreased at a slower rate than those stored at room temperature. Statistically significant changes ($p < 0.05$) in genistein levels were observed by the first month of storage. After storing for six months, the amount of Genistein left in cold storage (8 - 10°C) was 2.83 mg/kg which is a 57.76% reduction. Meanwhile, at room temperature, 1.87mg/kg of Genistein was left, showing a reduction of 72.09%.

Biochanin A (**Figure 2b**) also exhibited a similar trend in degradation like Genistein. The samples stored at a lower temperature retained more biochanin A while showing significant results ($p < 0.05$) so that it can be compared to room temperature samples. There was a declining trend in biochanin A content of 21.34% in cold conditions (8-10°C) and 34.05% in warmer conditions (28-30°C) during the archival period of the first four months. For the final four months, the cold temperature showed an increased degradation trend of 37.77% in Biochanin A while room temperature declined to 37.17%. By the end of the eighth month, the remaining biochanin A content was 1.83 mg/kg at room temperature and 1.20 mg/kg under cold storage.

These findings indicate that although both genistein and biochanin A levels declined over the eight-month storage period, cold storage (8 - 10°C) was more effective in preserving these bioactive compounds compared to room temperature (28 - 30°C).



(a) (b)

Figure 2. *The graph shows the effect of storage time and temperature on (a) genistein content and (b) biochanin content in peanut butter. Notes: The letters A, B, C, D, ... (Storage temperature) and a, b, c, d, ... (Storage time), different represent statistically significant differences (P < 0.05) and vice versa.*

3.2. Determination of 2,2-Diphenyl-picrylhydrazyl (DPPH) free radical uptake of peanut butter during storage.

The graph **Figure 3** shows the effect of peanut butter storage temperature on DPPH free radical uptake.

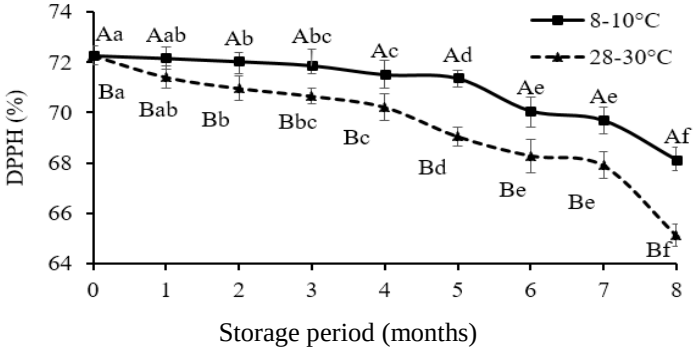


Figure 3. *DPPH Free Radicalization Activity of Peanut Butter Samples During Storage*
Notes: The letters A, B, C, D, ... (Storage temperature) and a, b, c, d, ... (Storage time), different represent statistically significant differences (P < 0.05) and vice versa

The DPPH percentage of samples stored at 8 – 10°C decreased more slowly than that of samples stored at 28 – 30°C, according to the results displayed in **Figure 3**. This difference was statistically significant ($p < 0.05$). In contrast to normal conditions, where it was approximately 69.05%, the ability to scavenge DPPH free radicals remained relatively stable during the first five months of cold storage, ranging from 72.28% to 71.37%. The product's compounds' oxidation resistance, however, drastically decreased in the last month of storage, reaching 68.12% in cold storage and 65.12% at room temperature by the eighth month. A number of intricate biochemical processes occur during storage. Fatty acids produce secondary compounds when they oxidize, whereas polyphenol compounds change into their corresponding o-quinones, which results in a reduction. A number of intricate biochemical processes occur during storage. Fatty acids oxidize to produce secondary compounds, and polyphenol compounds change into corresponding o-quinones, which decreases the product's ability to scavenge free radicals [15]. Furthermore, peanut butter is devoid of hydroxytyrosol and oleuropein, two common antioxidants present in olive oil. Oleuropein mainly scavenges lipid peroxyl radicals within the membrane, whereas hydroxytyrosol targets peroxyl radicals in water close to the membrane surface. Both of these phenolic compounds are very effective at neutralizing free radicals [16]. Studies using peanut butter and needle mushroom extract have also shown a similar pattern of decreasing DPPH free radical scavenging activity over time [17].

3.3. Investigation of variations in total lipid content, free fatty acid levels, and peroxide value in peanut butter throughout storage

3.3.1. Total lipid content (TF)

In fact, oxidation of lipid could be controlled by various parameters such as the type of peanut variety, water, water activity, processing conditions, storage time and temperature, as well as storage conditions and package conditions [15]. Change of the overall content of total lipids of the peanut butter upon 8-month storage is graphically demonstrated as shown in **Figure 4** and signifies that total lipid content became deteriorated steadily at both storage temperatures with statistical difference ($p < 0.05$). The first samples of peanut butter had a total lipid content of 51.15%. During storage for 8 months, the lipid content reduced at (8 - 10°C) and (28-30°C) to (4.10%) and (6.69%), respectively. Lipid oxidation took place at a high rate at normal temperatures compared to storage at low temperatures. This result is also shown in Khanh *et al.* (2023) [17] during the storage of peanut butter. This decrease is thought to be due to the effect of enzymes and oxygen from the atmosphere, where the presence of lipoxidase may be the major catalyst for the oxidation of unsaturated fatty acids, which alter the level of total lipids.

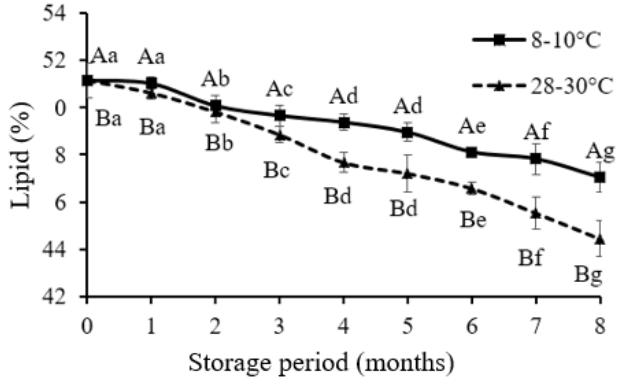


Figure 4. *Changes in lipid content in peanut butter products according to storage time at 2 different temperature conditions. Notes: The letters A, B, C, D, ... (Storage temperature) and a, b, c, d, ... (Storage time), different represent statistically significant differences (P < 0.05) and vice versa.*

3.3.2. Peroxide index (PV)

Figure 5 presented the effects of temperature and storage duration on lipid degradation, as measured by the peroxide index.

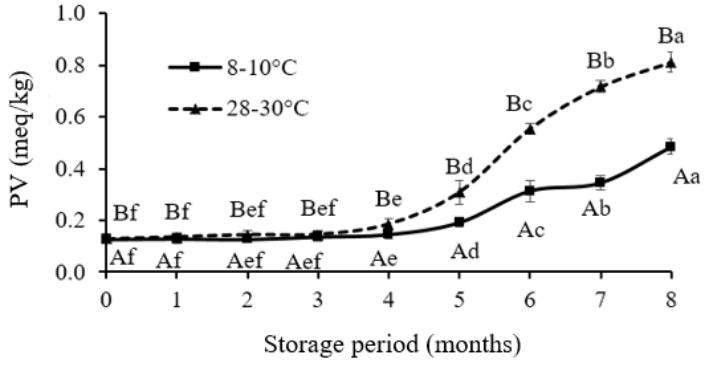


Figure 5. *Variation in the PV index of peanut butter over time under two different storage temperature conditions. Notes: The letters A, B, C, D, ... (Storage temperature) and a, b, c, d, ... (Storage time), different represent statistically significant differences (P < 0.05) and vice versa*

Figure 5 illustrated that peanut butter stored at 28 – 30°C exhibited an abrupt increase in peroxide value (PV) after eight months. The PV was 0.13 meq/kg initially, and increased to 0.8111 meq/kg after the eighth month, significantly different from the first four months ($p < 0.05$). Storage at lower temperatures slowed down the increase in PV. Particularly, at 8 – 10°C, PV before storage and the eighth month (0.4844 meq/kg) had a

significant difference ($p < 0.05$). For both storage temperatures, PV rose considerably from the fourth month. By the final month, PV in room temperature was 1.67 times higher than in cold storage. The findings indicate that lipid degradation varies according to storage temperature, and the warmer temperatures will accelerate lipid oxidation and nutrient loss. This agrees with the observations recorded in the study undertaken by Lee & Resurreccion (2006) [18].

3.3.3. Free fatty acid content (FFA)

Figure 6 illustrated the impact of storage duration and temperature on the free fatty acid content in peanut butter products.

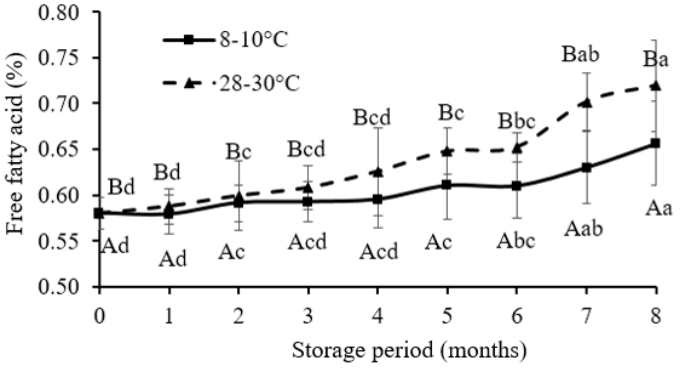


Figure 6. *Variation in free fatty acid content in peanut butter over time under two different storage temperature conditions. Notes: The letters A, B, C, D, ... (Storage temperature) and a, b, c, d, ... (Storage time), different represent statistically significant differences (P < 0.05) and vice versa*

The pattern of free fatty acid (FFA) content variation was similar to that of the peroxide value (PV) index. As shown in **Figure 6**, FFA content rose during storage, with a significantly different difference between the two temperatures ($p < 0.05$). Specifically, at 8 – 10°C, the FFA content kept rising from the 5th to the 8th month, to 0.61% and 0.66%, respectively. Meanwhile, in group 28 – 30°C, the rise ranged from 0.63% to 0.72%. The same growth of the FFA content has been confirmed by other researchers for investigations on the quality of cocoa butter during 65 days of storage in normal conditions [19]. The findings also indicate that FFA content degradation was strongest during the last four months of storage. Elevated FFA levels are adverse to the quality of butter, which deteriorates its flavor especially when stored under inappropriate temperatures [20]. Therefore, in this study, lower temperatures (8 – 10°C) were found to be better suited to store peanut butter compared to room temperature (28 – 30°C).

4. CONCLUSION

The bioactive compound content, lipid oxidation, and DPPH free radical scavenging capacity of peanut butter are influenced by storage temperature. A temperature of 8 – 10°C is considered more suitable for storage compared to room temperature (28 – 30°C). After eight months at 8 – 10°C, the remaining bioactive compound levels were: resveratrol (2.94 mg/kg), genistein (2.83 mg/kg), and biochanin A (1.83 mg/kg). The DPPH free radical scavenging capacity was maintained at 68.12%, while total lipid content (47.05%), peroxide value (0.4844 meq/kg), and free fatty acid content (0.66%) remained within acceptable limits, ensuring the quality of peanut butter throughout the storage period.

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