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Original Article

Real-Time Monitoring of Rancidity in Granola Foods: A Study Using Peroxide Value and Colorimetric Sensors

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ABSTRACT

One of the main causes of food quality and product degradation is lipid oxidation. It is known that a significant problem with the preservation of fatty acids in food products is lipid oxidation. Therefor this research explored the potential of granola products to develop rancidity by analyzing peroxide value (PV) and implementing smart packaging solutions. The PV method functions as a reliable quality index for the early stages of lipid oxidation. It was evaluated using six granola samples. The findings revealed a notable rise ($p < 0.05$) in PV from baseline levels (0.2 to 0.6 mEq O_2/kg) after storing the samples for two weeks at 32.5 ± 2.5°C and 27.5 ± 2.5% relative humidity, confirming the progression of rancidity. Furthermore, moisture content, which is a key determinant of food quality, showed a significant rise ($p < 0.05$) during storage, underscoring the absorption of moisture and its detrimental effect on shelf life. To enable real-time rancidity monitoring, smart packaging with natural dyes (anthocyanin and chlorophyll) was used. The chlorophyll-based indicators shifted from green to yellow, while anthocyanin-based indicators changed from red to brown when exposed to H_2O_2. Over the two-week period, significant decreases ($p<0.05$) in absorbance were recorded for chlorophyll (from 1.803 to 0.383) and anthocyanin (from 1.757 to 0.75). The color changes corresponded with increasing PV levels, offering a visual cue for granola quality decline. Using of smart labels on granola packaging successfully demonstrated real-time visual monitoring of product quality, consistent with earlier studies on colorimetric methods for detecting rancidity in food. The combined approach presents a practical alternative to conventional analytical methods, enhancing storage strategies and maintaining product quality.

Keywords: Oxidation detection, cereal product, smart packaging, natural pigments.

1 Introduction

Granola, a product made from dehydrated and granulated cereals, has low water activity. However, its shelf life is limited by chemical and physical changes, with environmental factors and composition influencing the rate of deterioration. Furthermore, relative humidity was shown to be the most important environmental component, while moisture content was identified as a crucial quality criterion [1]. Quantitative assessment of granola quality degradation is essential to estimate shelf life and develop innovative processes and packaging solutions [2]. In a study done by [3], various snack and cereal products, including peanuts, pork scratching, and a mixture of rolled oats and wheat from muesli and oatmeal, were evaluated to determine the relationship between storage humidity and stability against lipid oxidation.

The findings revealed that radical content is significantly influenced by relative humidity. Additionally, the study showed that while the oxidative stability of peanuts exhibits moderate fluctuations, the oxidation rate of oatmeal lipids is highly dependent on moisture levels.

Lipid oxidation is a significant factor in the degradation of food quality and the deterioration of food products. It is widely recognized as a major challenge in preserving fatty acids in food. Although lipid oxidation products are commonly present in diets, their types and concentrations vary significantly. Even at relatively low levels, these substances can severely compromise

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the quality of certain foods and reduce the shelf life of others. Oxidative changes often lead to rancidity, which is characterized by unpleasant flavors, color changes, and a decline in nutritional value. Additionally, these changes can produce harmful compounds that pose risks to consumer health [4]. Maintaining oxidative stability during storage is vital for preserving the quality of food products. Unavoidable oxidation reactions, particularly those impacting the lipid components of foods, lead to nutritional and sensory degradation, substantially shortening shelf life [5].

As a result of escalating concerns regarding food safety and public health, significant efforts have been directed toward developing effective, reliable, non-destructive techniques for assessing the freshness of various food types [6]. In modern food systems, packaging technologies are vital in preserving and delivering food products. Consequently, the development of smart packaging solutions capable of providing real-time, reliable information about food quality in a user-friendly format has gained increasing attention [7]. For example, a package integrated with colorimetric sensors can rapidly and precisely detect key food safety and quality parameters [8]. These colorimetric sensing systems typically consist of a solid matrix and a pH-sensitive dye indicator [9].

As food spoils due to microbes and chemicals, compounds like amines, ammonia, hydrogen sulfide, carbon dioxide, and organic acids are released. such compounds are linked to changes in the food's pH, which can be detected using in-package indicators with pH-sensitive compounds [10]. Most pH indicators studied for this purpose have utilized artificial dyes and carcinogenic substances like bromothymol blue, bromocresol green, and bromocresol purple [11]. Natural and safe pH-sensitive colors for food packaging, such as anthocyanins and betacyanin, are being considered [10].

It was mentioned by [12] that chlorophyll is a natural pH-responsive dye due to its structural alterations under varying pH conditions, resulting in a spectrum of colors. For immobilizing natural colorants, polymer substrates, or matrices are frequently utilized, which include polysaccharides (e.g., cellulose, pectin, starch, alginate, carrageenan, agar, pullulan, chitosan, and their derivatives) and proteins (e.g., corn zein, collagen, wheat gluten, and gelatin).

With the growing popularity of granola products across different regions and countries, ensuring their safe preservation during distribution and after opening remains a significant challenge. There is also a noticeable gap in the investigation of shelf life and rancidity processes in these items and in the application of smart packaging. This study aimed to determine the effectiveness of peroxide value (PV) measurements and smart packaging sensors in detecting and monitoring rancidity in granola products under controlled storage conditions (32.5 ± 2.5°C and 27.5 ± 2.5% humidity). It was predicted that granola products would show increased rancidity at the specified temperature and humidity levels and that PV measurements and smart packaging techniques would effectively detect and monitor it. The study used natural materials instead of synthetic ones to ensure product safety. The selected storage conditions of relative humidity and optimal temperature replicate ideal preservation conditions for granola.

2 Material and methods

2. 1 Materials

Chard and the dried calyces of Roselle (*Hibiscus sabdariffa L.*) were utilized to prepare extracts. Wheat gluten, obtained commercially, was used to isolate gliadin. Six different granola brands (labelled S1 to S6), each with three replicates, were purchased from local markets in Sulaimani City, Iraq.

2.2 Methodology:

2.2.1 *Peroxide Value (PV)*

PV (in meq/kg) was determined according to the AOAC Official method 965.33 Peroxide Value in Oils and Fats for the oil analysis [13]. The granular oils' PV was measured initially (at zero time) and after a two-week storage period.

2.2.2 *Anthocyanins Extraction*

Anthocyanins were extracted from dried Roselle calyces by macerating them in a solution of citric acid monohydrate and sodium bicarbonate using a water bath maintained at 70.2°C for 60 minutes. After extraction, the solution was promptly cooled to 32°C and filtered [14].

2.2.3 *Anthocyanin Content*:

The anthocyanin content in the extracted calyces of Roselle was determined following a previously reported method [15]. The absorbance was measured using a UV-Vis spectrophotometer at 400 nm and 700 nm wavelengths. Based on Equations 1 and 2, the anthocyanin content was calculated and expressed in mg/mL:

$$\text{Total anthocyanins} \left(\frac{\text{mg}}{\text{ml}}\right) = \text{Total dry weight/total anthocyanin was expressed} \quad \text{Eq.1}$$

$$\text{Total dry weight}/\ 60\ \text{ml} = \text{dry weight} * \text{volume made up} * 60/\ \text{ml juice taken} \quad \text{Eq.2}$$

2.2.4 *Chlorophyll Extraction*:

Water was used as a solvent in a straightforward mechanical-thermal extraction process to extract chlorophyll from chard leaves. A leaf-to-water mass ratio of 1:2 was used for the extraction process. The mixture was heated for approximately 2 minutes, filtered, and the resulting extracts were dried in the dark at room temperature. The extracted chlorophyll was dissolved in 1 mL of a 70% ethanol solution in a conical flask wrapped with aluminum foil. The absorbance of the total chlorophyll extract was measured at wavelengths of 645 nm (D645) and 663 nm (D663) using a UV-Vis spectrophotometer. Finally, the total chlorophyll concentration (chlorophyll a and b, expressed in mg/L) was calculated according to Equation 3:

$$\text{Total chlorophyll content} = 20,31\text{A}645 + 8,05\text{A}663 \quad \text{Eq.3}$$

2.2.5 Gliadin extraction:

Gliadin was extracted from gluten using 60% ethanol, according to [16]. Gliadin-rich fraction was isolated by centrifuging the mixture at 9000 rpm for 15 minutes at 25°C. Following centrifugation, the supernatant containing dissolved gliadin was collected, and ethanol was evaporated to separate the gliadin from the solvent.

2.2.6 *Preparation of the colorimetric indicator sensor*:

To prepare the colorimetric indicator, 1 mL of a 0.1% gliadin-glycerol solution was spread onto a clean surface. Extracted anthocyanins and chlorophyll were added, followed by enzymes (α-amylase and glucose oxidase), all at a uniform concentration of 5%. A starch substrate was then incorporated at 10% (w/v). The indicator sensors were dried at 25–30°C under 33–35% relative humidity for 24 hrs.

2.2.7 *Relative humidity determination*:

The relative humidity was assessed using a digital thermometer/hygrometer (Mebus Sender/Aussender 40424).

2.2.8 Fat Extraction:

The fat extraction method followed the AOAC method 922.06 [17].

2.2.9 Determination of Moisture

The moisture content percentage of selected granola samples was determined before and after two weeks of storage, following the AOAC Official Method 934.06 [17].

This study constitutes Phase 1 of our preliminary investigation, employing varied parameters (temperature, humidity, duration) and testing multiple smart sensors. A two-week experimental period—aligned with the assumed consumption timeframe for a standard box of granola products—was implemented in this research.

3 Statistical Analysis:

Results are reported as percentages and mean ± standard deviation (n = 3). For statistical analysis, Origin Pro 9.0 software (OriginLab Corp., USA) was utilized. A one-way ANOVA was applied to all datasets, with statistical significance defined at $p < 0.05$. This method identified significant differences between experimental groups.

4 Results and discussion

This study assessed granola's susceptibility to rancidity using two methods. One method involved quantifying peroxide value (PV, mEq O_2/kg), while another method utilized a smart packaging technique. As demonstrated by [18]. PV functions as a quality index capable of identifying early-stage lipid oxidation. PV values were recorded as follows: 0.47, 0.40, 0.20, 0.40, 0.60, and 0.40 mEq O_2/kg for samples 1 to 6, respectively. However, after 2 weeks of storage, the PV increased significantly ($p < 0.05$) across all samples. This upward trend under experimental conditions (32.5 ± 2.5°C and 27.5 ± 2.5% humidity) indicates that oxidative rancidity progressed during storage. These results underscore the susceptibility of the granola product to rancidity under the tested environmental conditions.

The moisture content of all granola samples was determined under storage conditions, as it is a critical quality parameter for assessing rancidity and oxidation [1]. Moisture content analysis also provides insights into variations in moisture levels during storage, which is essential for mitigating rancidity and maintaining product quality. The measured moisture content values for each sample are presented in Table 1. Under the tested storage conditions, all samples exhibited a statistically significant increase in moisture content ($p < 0.05$).

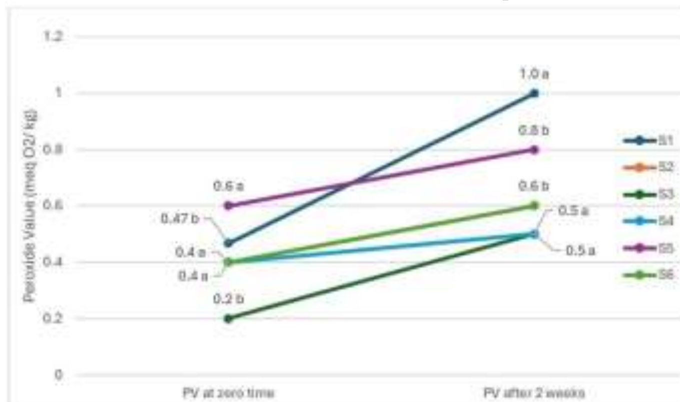

Figure 1: Peroxide value for different samples of granola, at the initial time, after two weeks of storage, at temperature (25-30) C, and humidity (30-36) %. Values are expressed in mean ± standard deviation (n=3). Superscript lowercase letters indicate significant differences at p<0.05 by Tukey's multiple comparisons tests.

All samples demonstrated increased moisture content after 2 weeks of storage, but some did not display significant changes ($p<0.05$). The magnitude of moisture gain varied significantly among most samples under storage conditions, with sample S3 demonstrating the most pronounced increase ($p < 0.05$).

This comparative analysis demonstrated that storage conditions significantly influenced the moisture content of granola samples. The observed moisture gains over the 2-week storage period indicated that the samples were prone to moisture uptake, likely influencing their susceptibility to quality degradation and reduced shelf stability.

In addition to the PV method for detecting rancidity in the granola material, the smart packaging was used for real-time quality monitoring of the granola structure regarding rancidity when inserted into the granola package during storage at 32.5± 2.5°C and 27.5 ±2.5% humidity. Smart packaging incorporating natural dyes (anthocyanin and chlorophyll) was employed. Once the ambient humidity reached 25–30%, starch hydrolysis was initiated, driven by the enzymatic activity of α-amylase on the α-1,4 glycosidic bonds within the starch polymer. This process increased glucose availability, which was subsequently oxidized by glucose oxidase into gluconic acid and hydrogen peroxide (H_2O_2). Exposure to H_2O_2 triggered a colorimetric response in the chlorophyll-based indicator, transitioning its visible color from vibrant green to yellow. In contrast, anthocyanin changed from red to brown [19], altering the color of the pigment sensor (per 2).

The colorimetric absorbance values for two different colorimetric indicator sensors are presented in Figure 2. One sensor contains chlorophyll (green bar), and the other contains anthocyanin (red bar), with measurements taken at two time points: initial (zero time) and after 2 weeks. These absorbance values were determined to assess the pigment concentration, which may reflect stability and potential degradation over time. Both chlorophyll and anthocyanin initially exhibited elevated absorbance values, indicating a strong pigment presence. After 2 weeks, the absorbance of both pigments decreased significantly ($p<0.05$), with a more pronounced decline in the chlorophyll sample (from 1.803 to 0.383) compared to anthocyanin (from 1.757 to 0.75). This decrease in absorbance suggests degradation or a decline in pigment concentration over time.

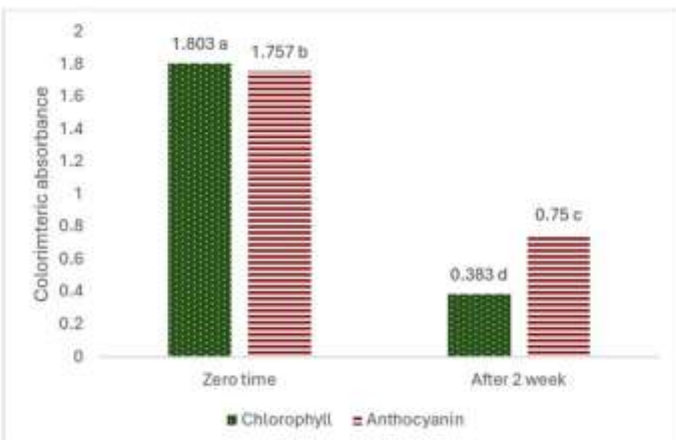

Figure 2: Colorimetric absorbance of the chlorophyll and anthocyanin smart label. At the initial time, after two weeks of storage, at temperature (25-30) C, and humidity (30-36) %. Values are expressed in mean ± standard deviation (n=3). Superscript lowercase letters indicate significant differences at p<0.05 by Tukey's multiple comparisons tests.

Figure 3 shows the images of the smart labels, clearly demonstrating changes in color corresponding to the duration of granola storage. Initially, the chlorophyll smart label displayed green, signifying high-quality granola. As storage time progressed, the green hue of the smart label faded, correlating with the increasing peroxide value (PV) due to the rancidity of granola fat. Similarly, the purple-reddish color of the anthocyanin smart label diminished over the storage period, indicating the effect of rising PV throughout the storage duration.

Figure 4 illustrates the application of the smart label on the granola package. Incorporating chlorophyll and anthocyanin pigments, this label was enclosed in a specialized cover and affixed to the packaging. These findings align with research conducted by [20], which demonstrated the efficacy of a colorimetric method in accurately detecting rancidity in intermediate-moisture products like cake. By implementing this approach, one can visually monitor freshness and best-before dates. The same technique was effectively utilized by [21], where they developed a colorimetric pH dye-based indicator using bromothymol blue and methyl red to monitor rancidity in infant milk powder stored at 30°C, 40°C, and 50°C. The colorimetric indicator directly correlated with peroxide value (PV), visualizing product freshness by signaling oxidative spoilage. Complementing this approach, a recent study developed a chitin-based colorimetric sensor to detect aldehydes—key markers of lipid oxidation in edible oils. The sensor was fabricated by integrating Congo red and hydroxylamine sulfate into TEMPO-oxidized chitin films, which underwent a distinct chromatic transition from red to blue upon aldehyde detection. This system demonstrated high efficacy in evaluating the oxidation state of commercial sunflower oil samples, offering a cost-effective and user-friendly alternative to conventional analytical methods such as titration or chromatography. Both studies highlight the potential of colorimetric sensors as accessible tools for real-time quality assessment in food systems [22].

Smart label pigments	Initial time of storage	Post two weeks of storage
Chlorophyll		
Anthocyanin		

Figure 3: Visual images of intensity color changes of smart label pigments (Chlorophyll and Anthocyanin), corresponding to the level of rancidity of granola at a temperature range of (25-30) C, and humidity (35-36) %, during the initial time, 2 weeks later of storage time.

Figure 4: Application smart label on the granola package.

Overall, these results demonstrated the efficacy of integrating PV measurements with colorimetric indicator sensors for thorough monitoring of rancidity in granola items, providing insights for enhancing storage practices and maintaining product quality.

Conclusion

The present study confirmed that granola products are highly susceptible to rancidity under controlled storage conditions (32.5 ± 2.5°C and 27.5 ± 2.5% humidity), as demonstrated by significant increases in peroxide value (PV) and moisture content. PV served as a robust indicator of lipid oxidation, with all samples exhibiting marked increases over the two-week storage period. Concurrently, the rise in moisture content underscored the material's hygroscopic nature, which compromised product quality and shelf stability. Integrating smart packaging incorporating anthocyanin- and chlorophyll-based natural dyes effectively enabled real-time rancidity monitoring, as evidenced by colorimetric changes in the indicators correlated with rising PV levels. To advance these findings, future research should investigate the impact of variable temperatures, humidity levels, and extended storage durations on rancidity progression, to evaluate the long-term efficacy of smart packaging systems under diverse environmental conditions.

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