Colorimetric indicators for revealing oxidation in certain kind of maize oil and whole powder milk

[1]Omer Abdulqader Abdalla

[1]Department of Food Science and Quality Control, College of Agricultural Engineering Science, University of Sulaimani, Iraq

*Corresponding author's email: omer.abdulla@univsul.edu.iq

Abstract:

A colorimetric indicators mixed natural pigments – enzymes –substrate based indicator to oxidation reaction of powder milk formula and corn oil was described. Milk powder stored in 25-30 ºC was covered and non-covered samples for 1 week. Also, corn oil was detected after 1week storage at 25-30 ºC after addition 1microliter of rancid oil. The addition of 1% glucose oxidase (270 U/0.01 g), 1 % alpha-amylase (117 U/0.01 g), and 1% natural pigments significantly enhanced the sensor's sensitivity to oxidation reaction. It was found that the absorbance of pigments was decreased after measured by spectrophotometer as a consequence of enzymatic reaction when glucose oxidase reacts with glucose and convert into gluconic acid and hydrogen peroxide. The highest level of absorbance was noticed in green colormeteric indicator with unsealed sample of powder milk 0.304 while the lowest value was obtained in yellow colormeteric indicator beta-carotene was 0.024 with unsealed powder milk sample. The concentrations of acetic and hexanal acid raised as the milk powder's moisture content rose, and they were liberated from oxidation and hydrolytic reactions across the storage period. In a similar vein, the oil samples with the green color indication had the highest absorption0.225 , while the beta-carotene color indicator, which is yellow, had the lowest value0.073. Anthocyanin, chlorophyll, and beta-carotene exhibit color changes in reaction to volatile chemicals, correspondingly going from red to pale red, green to yellow, and yellow to white on the colorimeter.

Key words: colormeteric indicators**,** Glucose oxidase, Alpha amylase and oxidation

Introduction:

One of the most important problems with this product is the presence of unnatural flavors in the milk powder formula during storage. Flavors not present in powdered milk formula usually result from hydrolysis and oxidation of fats. Lipid oxidation occurs through a three-stage free radical chain reaction process: initiation, propagation, and termination. Unsaturated fatty acids served as catalysts for the creation of free radicals during the initiation phase. UV light, photo sensitizers, visible light, free radicals from other sources, ionizing radiation, metals, household chemicals, and heat are examples of common initiators. Proxy radicals would be created when free radicals and oxygen reacted. Hydro peroxides are the main oxidation products during the propagation stage of a free radical, and a proxy radical extremely selectively absorbs hydrogen from nearby lipid molecules to generate them [1]. The secondary lipid oxidation products (alkenes, alkenes, aldehydes, ketones, etc.) that cause a change in the flavor and odor of food products are formed when lipid free radicals terminate to produce non-radical products [2].

Hexanal may be a helpful indicator of lipid oxidation in this situation, however hydrolytic

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rancidity, often referred to as lipolysis flavors, is the term used to describe the off-flavors in rancid dairy products that result from the release of free fatty acids (FFA) from milk due to the action of lipases. The breakdown of milk lipid, triglyceride, is what causes hydrolytic rancidity. Lipase catalyzes this hydrolysis, which results in the production of free fatty acids and involves both indigenous milk enzyme and enzymes from microorganisms [3].

The majority of techniques to identify rancidity reactions are time-consuming and difficult to comprehend in lab settings. An effective substitute for tracking rancidity in milk powder formulae is the colormeteric indicator. This method has demonstrated potential for use in numerous products. A unique colorimetric mixed-dye-based indication for tracking the freshness of intermediate-moisture dessert spoiling, as well as a colorimetric dye-based sensor and indicator for monitoring fish spoilage based on the presence of total volatile basic nitrogen (TVB-N) a newly created colorimetric mixed-dye indicator that detects the freshness of desserts that deteriorate at an intermediate moisture level [4].

Introduced an aldehyde indicator pad used in chemical industry applications for quick aldehyde detection. It was demonstrated that this indicator pad changed visibly from yellow to red in response to glutaraldehyde. When glutaraldehyde was added, the aldehyde indicator's color changed as the pH decreased. In order to monitor the rancidity reaction of oxygen-sensitive dairy products, a new rancidity indicator was created to monitor the formation of acetic acid and hexanal from hydrolysis and oxidation, respectively [5].

Corn oil is one of the vegetable oils that are highly recommended for use as a cooking medium for humans because of its high level of polyunsaturated fatty acids and several health benefits [6]. Triglycerides, which are derivatives of fatty acids, exhibit unstable chemical structures when exposed to extreme environmental factors including air, light, and continual high temperatures. Owing to their high fat content, fatty acids and their derivatives are readily oxidized by chemicals, which shortens their shelf life and imparts a rotten taste [7]. Consequently, the rancidity of oils significantly reduces their market value and nutritional content, potentially resulting in a loss of profit [8]. Colormeteric indicators of anthocyanins, beta carotene and chlorophyll can provide rapid qualitative information through visual colorimetric changes caused by the structural change of the pigment intensity based on enzymes glucose oxidase and alpha amylase – starch.

The current work's aims included evaluating the quality of corn oil and milk powder formula stored at both ambient and increased temperatures, creating a colorimetric indicator to track rancidity reactions, and calculating the products' respective shelf lives.

Materials

Dehydrated calyces, chard, carrots, whole powdered milk and maize oil were commercially available in the local market in Sulaimani, Iraq, along with Roselle (Hibiscus sabdariffa var. sabdariffa). Additional ingredients were acquired from AVOCHEM Ltd. Chemical Co. (Cheshire, Sk116PJ.U.K.) and included soluble starch, ethanol absolute 99 % (C_2H_6O) (Scharlau, Spain), petroleum ether and glacial acetic acid (Chem-Lab, Belgium), glucose oxidase % (activity 270U/0.01g), and alpha-amylase 1% (117U/0.01g) enzymes. (Lab Tech, China) 250W heating mantle. 0.1-2 µL ONE SeriesTM micropipette. UV-Vis spectrophotometry (Jenway -7205-the UK by Cole-Parmer Ltd.) was used to apply scanning pigments.

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Anthocyanins Extraction

The calyces underwent hand separation, distilled water washing, and draining. The calyces were then dried at 45˚ C in an oven dryer (Ufp500/Memmert). Before being used, the dried calyces were placed in a black PE bag, double Polythene (PE) pouches, and preserved in a deep refrigerator at -18°C. Prior to examination, the calyces were ground using a Mulino mixer blender using a variety of modifications from the methodology in [9]. The powder is extracted aqueously using sodium bicarbonate and citric acid monohydrate, respectively. For 20, 40, and 60 minutes, the Roselle powder was extracted in a water bath set at 70.2% Celsius. The conical flasks were taken out of the heat treatment, warmed to (32°C), and then filtered right away. In the first series of tests, the solid-liquid ratio was 1:10. The second set of experiments involved using a pH 7 solvent and extracting the material for 20 minutes at 70.2 °C. Similarly, the solid-to-liquid ratio shifted from 1:50 to 1:100 to 1:150. The process described in [9]. Was used to carry out the aqueous extraction. Roselle powder was soaked in distilled water and ground in a mortar and pestle to extract all of the anthocyanins. Until the residue was colorless, the procedure was repeated. The volume of a conical flask was 50 ml. After that, the extract was filtered in order to get it ready for further examination.

Beta carotene extraction

Conditions of extraction: Root slices (width 2 mm, length 1 cm) were employed in accordance with the process outlined in Slovak Technical Standard 56 0053 [10]. The extraction yield of carotenes was tested using 96% ethanol at different temperatures (20–25°C). 40 g of chopped carrot samples were added to 240 ml of 96% ethanol and 10 ml of distilled water, and the mixture was put inside a beaker covered with aluminum foil. At 60 degrees Celsius, carrot slices were removed from a water bath and shaken every ten minutes. Ten milliliters of the sample were taken out and mixed with ten milliliters of petroleum ether after each extraction hour.

Chlorophyll Extraction

In Sulaimani, Iraq, chard leaves were purchased from a nearby supermarket. The leaf extract was made via a straightforward mechanical-thermal extraction process with water as the solvent. The extract was separated with the aid of filtering. The study's 1:2 mass ratios of 50 grams of leaves and 100 milliliters of solvent were heated for two minutes, after which the chlorophyll was separated and allowed to dry at room temperature in a dark location for the entire night.

One milliliter of a 70% ethanol solution containing 100 mg/ml of chlorophyll-extracted pigment. To protect it from light and preserve the stability of the process, the chlorophyll was extracted in a conical flask coated in aluminum foil. Despite its stability, heat, light, oxygen, acid, and enzymes quickly break down chlorophyll when it is extracted from plant tissue [11].

Natural pigments Extracts Scanned by spectrophotometer:

The best anthocyanin absorbance at 494 nm was found with a spectrophotometer. It was established what the ideal chlorophyll absorption was at 666 nm. Using a UV-Vis spectrophotometer (Jenway -7205-UK by Cole-Parmer Ltd.) set to 450 nm for beta-carotene pigment, the extract's absorbance was measured.

All of the dyes were scanned from 350 nm to 700 nm in order to determine the Lambda Max, or ideal absorption wavelengths, for each extracted dye. since the absorbance values and

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color intensity of the extracted natural dyes were the primary focus of the study's results, a scan was required to identify the optimal absorbance for each of the extracted pigments and to detect precise and specific absorbance.

Indicator label fabrication

Two milliliters of the solution were added to a clean, smooth, 10 mm-diameter plastic petri dish lined with filter paper to generate each label. Based on the predicted anthocyanin content (0.1 percent solution), a specific amount of powdered anthocyanin extract was then added. Beta-carotene and chlorophyll pigments were added at amounts of 1% and 1%, respectively. The enzymes glucose oxidase, alpha amylase and substrate (starch) were also added to the compositions at the same concentration (1%).

StatisticalAnalysis

Three packets of the milk powder and maize oil samples were taken. Each sample analysis was conducted three times, with a total of nine determinations per test condition, to determine the colorimetric indicators intensity of the pigments in all three samples. For each sample in the statistical analysis, the average of the three outcomes was employed. Each data point was examined using the Duncan test at the 5% level using the statistical tool XSTATE, 2016

Results and Discussion:

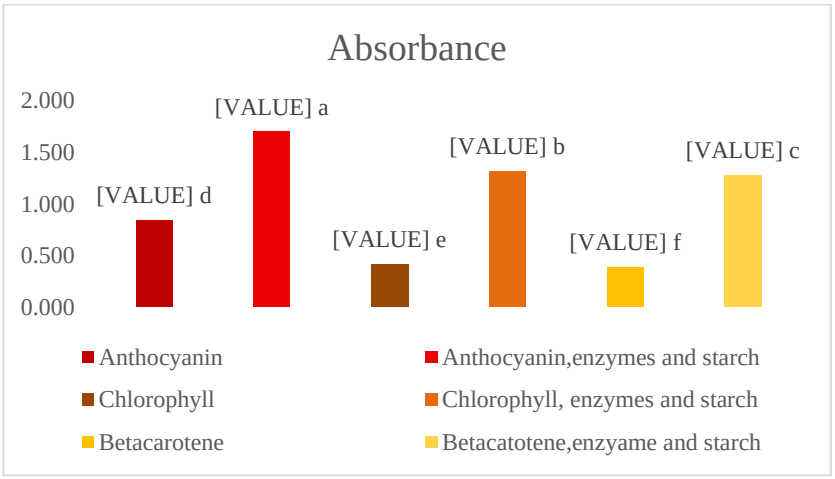
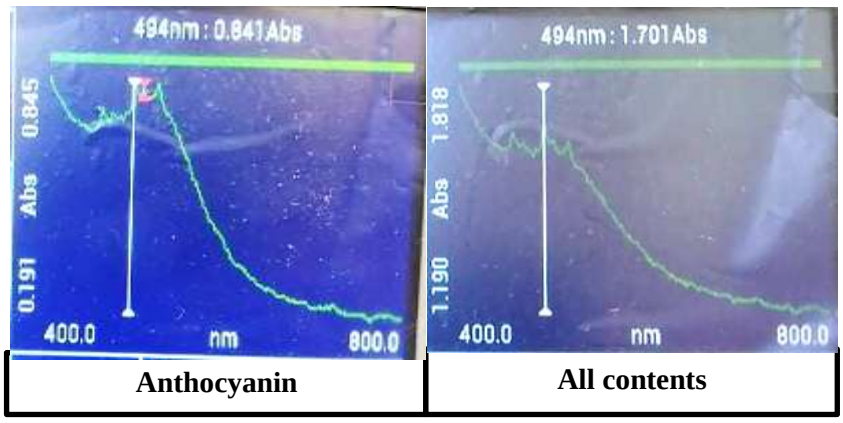


Figure.1 natural pigments (Anthocyanin, chlorophyll and beta –carotene extracted and colormeteric indicators (Absorbance and concentration)



Anthocyanin | **All contents**

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Figure.2 Natural pigments extracted and colormeteric contents with enzymes alpha, glucose oxidase and starch scanned by spectrophotometer at 494nm

The first stage of the experimental protocol is illustrated here: anthocyanins serve as natural colorimetric indicators within enzymatic systems. Anthocyanins were mixed with alpha-amylase, glucose oxidase, and starch, then the resulting solution underwent spectrophotometric analysis.

The experimental configuration simulates the detection of oxidative stress. Alpha-amylase cleaves the 1,4-glycosidic bonds of starch, releasing glucose. Alpha amylase was added to increase glucose oxidase efficiency through hydrolyses 1,4 glyosidic linkages between glucose units in starch chain and that leads to make glucose available to glucose oxidase and then conformed into gluconic and hydrogen peroxide the latter impact on intensity of dyes [12]. When combined with enzymatic amplification—using alpha-amylase to release sugars and glucose oxidase to produce hydrogen peroxide and that was mentioned by [13]. The colorimetric indicators yield real-time colorimetric shifts that are easy to read with the naked eye.

This glucose then acts as the substrate for glucose oxidase, which transforms glucose into gluconic acid and hydrogen peroxide ($H_2 O_2$) [14]. The subsequent production of $H_2 O_2$ oxidizes the anthocyanin pigments, generating distinct and quantifiable changes in absorbance and that was compatible somewhat with findings of [15]. Additionally, $H_2 O_2$ can bleached the natural extracted pigments as the results shown in the bar charts and that was consistent with results by [16]. The enzymatic pathway described culminates in the degradation of the pigments, providing a highly sensitive readout for oxidative stress. Starch hydrolysis occurs by the enzymatic action of α-amylase on the α1,4 glyosidic bonds in the starch polymer. And, the results shown there are significant differences between each pigment with same pigments with all contents of enzymes and substrate was latter increased the concentrations of absorbance after measured by spectrophotometer at 494 ,666 and 450 nm respectively. The bar chart illustrates increasing the all contents with anthocyanin approximately for double value compared to anthocyanin was 0.841to 1.701and that as result of adding enzymes and starch contents to the mixture and later the occurrence of the reaction mentioned above. As well as, the outcomes in the figure.1 emphasized rose the levels of absorbance for both green and yellow mixtures contents roughly to triple value from 0.415 to 1.316 and 0.386 to 1.279 respectively.

This observation confirms the findings of [6,18], who demonstrated that natural phenolic pigments are susceptible to degradation upon oxidative insult, thereby offering a clear visual indication of oxidation. When comparing the absorbance of all pigments with their all mixture contents (enzymes and

starch) respective concentrations of anthocyanin, chlorophyll and beta-carotene, (1.701, 1.316, and 1.279) to natural dyes separately, there are notable variances and that was somewhat consistent with findings by [17].

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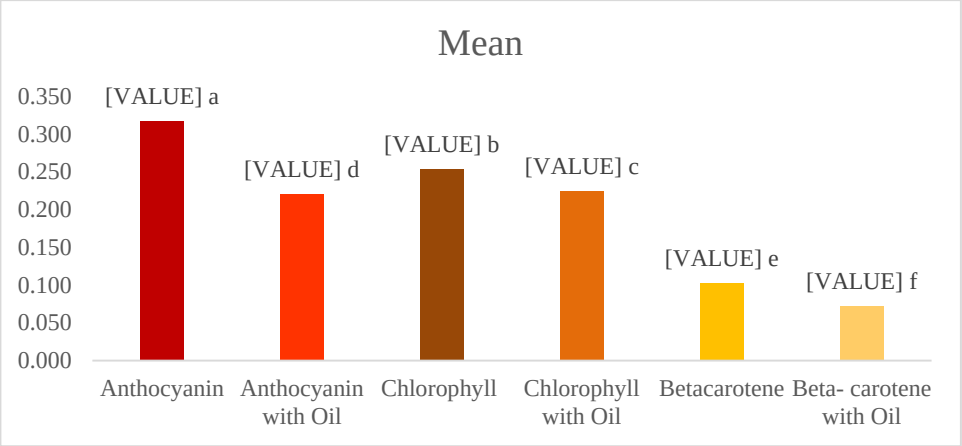

Figure.3 colormeteric indicators with maize oil samples compared to natural dyes (Absorbance and concentration)

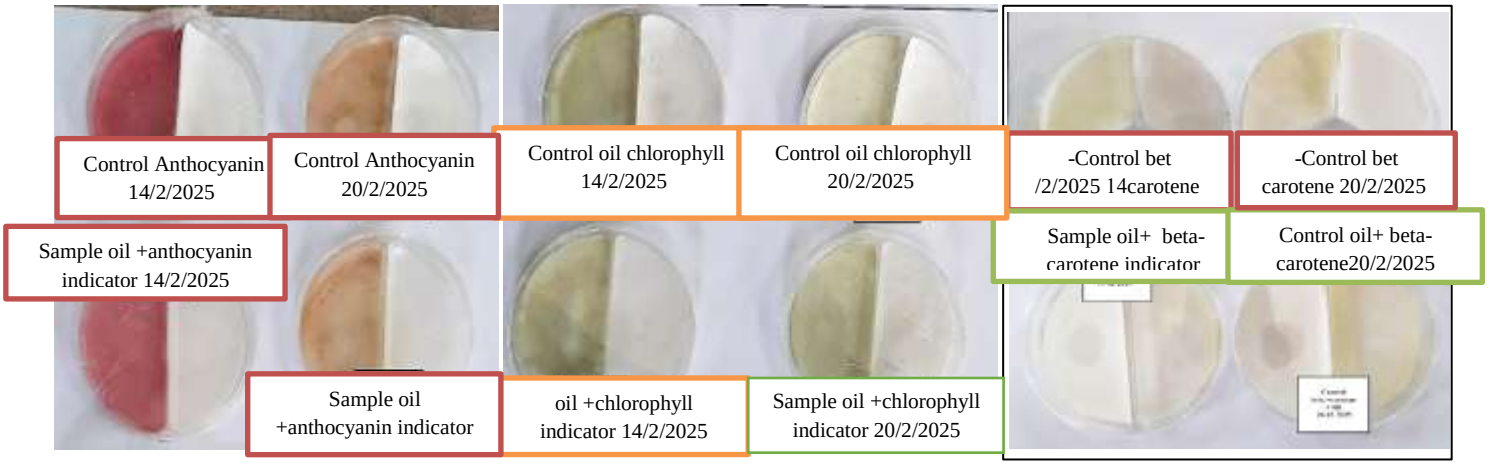

Figure.4 Colormeteric indicators after 1-week storage after addition 1microliter of rancid oil.

The bar chart in the figure 3 presents the spectrophotometric behavior of the pigment-enzyme complex following the addition of 1 µL of rancid oil—an experimental model for lipid oxidation—and subsequent storage and that was a compatible with outcomes by [18]. The lipid radicals and secondary oxidation products released during the rancidification process function as catalytic pro-oxidants and thus accelerate the destruction of the colored pigment [19].

After a week of storage, the spectrophotometric data revealed a marked decline in absorbance, correlating with the expected oxidative degradation trajectories. Lipid oxidation by a free radical chain reaction process involving three stages: initiation, propagation and termination.

During the initiation stage, an initiator used unsaturated fatty acids to create free radicals. UV light, photosensitive substances, visible light, ionizing radiation, metals, household chemicals, heat, and radicals from other sources are examples of common initiators. A proxy radical would be created when oxygen and free radicals reacted. Hydro peroxides are created when a proxy radical very specifically extracts hydrogen from nearby lipid molecules and generating hydro peroxides, the primary

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oxidation products in the propagation, as well as new free radicals.

The accumulation of malondialdehyde and lipid hydro peroxides, the survey of primary oxidation products, is known to attack chromophoric structures and the timing of the fading corresponds to the expected kinetics of these secondary products: which are oxidation products (alkenes, alkenes, aldehydes, ketones, etc.), that are responsible for a change in taste and odor of food products. Hexanal may be a helpful indicator of lipid oxidation in this situation, nevertheless hydrolytic rancidity, often referred to as lipolysis flavors, is the term used to describe the off flavors in rancid products that result from the release of free fatty acids (FFA) from due to the action of lipases. The hydrolytic decomposition of oil produces hydrolytic rancidity and that was mentioned in [20].

The pronounced decline in pigment levels therefore demonstrates the system's sensitivity to oxidative oil spoilage. As shown in figure .3 pigments in colorimetric indicators response were decreased significantly in all colormeteric mixtures compared to all natural extracted dyes and that were expressed by absorbance values. The values of red indicator with oil sample was decreased significantly compared to anthocyanin pigment was 0.220 while, green indicator was a slightly reduced in absorbance with corn oil sample was noted at 0.225 as a result of response the mixture to oxidation. The figure.3 illustrate the gradual impact of oxidation on the intensity of colorimetric indicator. The lowest concentration of beta-carotene was 0.073, which resulted from a reduction in the pigment indicator's intensity with a corn oil sample after seven days of storage. The color of colorimetric indicators has changed and were shafted the colors of colorimetric indicators from red to pale red, green to yellow and yellow to pale yellow respectively. furthermore, there is an apparent decrease in absorbance value; this outcome is comparable to research showing that colormeteric films react to oxidation as a series of rising peroxide values in oil following progressive oxidation-induced spoiling [5, 21].

Rancid oils are enriched in lipid hydro peroxides, which are established accelerants of anthocyanin destruction. The linear relationship between oil rancidity and pigment loss corroborates our hypothesis that the sensor quantitative detects oxidation via colorimetric indicator shifts When compared to the control sample, the anthocyanin mixture's color changed noticeably from red to pink and gradually for pale color due to the oxidizing reagent H2O2 as result of reaction enzymatic reaction with gluconic acid, and its concentration was approximately reduced in half. Furthermore, the absorbance measured at 666 nm declined in the control from 0.535 to 0.235, which was related to the bleaching of chlorophyll brought on the green color of the chlorophyll mixture with uncovered milk powder sample was also impacted by H2O2 and oxidation thus changed to yellow cause of responded and sensitivity the mixture for oxidation and, this outcome somewhat compatible with results studied by [22] was colorimetric film sensor showed highly reactive for rancidity of cake samples and oxidation by changing them color from dark color to pale colors .

The outcomes are consistent with the conclusions of [8,22], who documented that edible oil rancidity is marked by rising peroxides and aldehydes; these can be reliably assayed by established TBARS protocols or by monitoring pigment deterioration under controlled conditions. The combination of substrate and enzymes greatly affects how intensely color films change. Furthermore,

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when the color characteristics of the active film are analysed, it is found that the color of the chlorophyll film changes from green to yellow, while the color of the beta-carotene film changes from yellow to pale yellow, and the color of the anthocyanin film changes from red to brown. These changes can be used to identify the rancidity reaction of cake fat. Also, showed that after three weeks of storage, the rancidity response in cake samples significantly decreased the intensity of anthocyanin, beta-carotene, and chlorophyll color films. All treated samples showed a decrease in color pigment intensity compared to the control and that was clearly the outcomes of absorbance showed in the figure. 5 provides impact of enzymatic reaction of the colorimetric indicators and oxidation reaction how impacted on unsealed samples. In particular, the beta-carotene yellow indicator was reduced significantly in uncovered milk powder sample from 0.099 to 0.024 was the lowest value was recorded

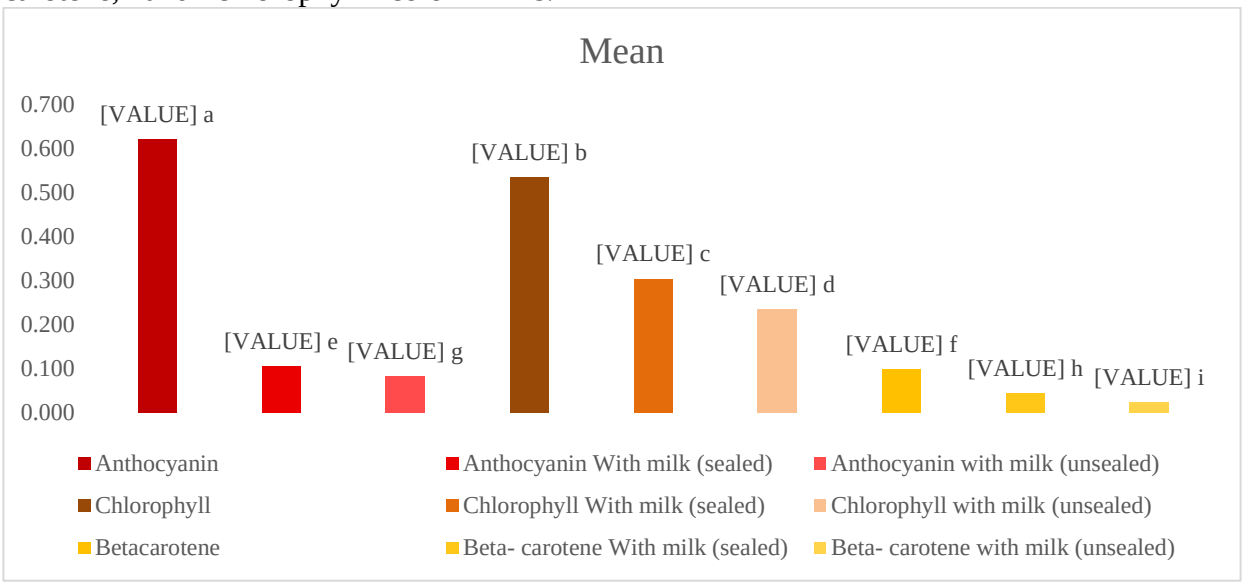

Figure.5 Absorbance levels of natural pigments anthocyanin chlorophyll, beta-carotene and milk powder sealed and unsealed

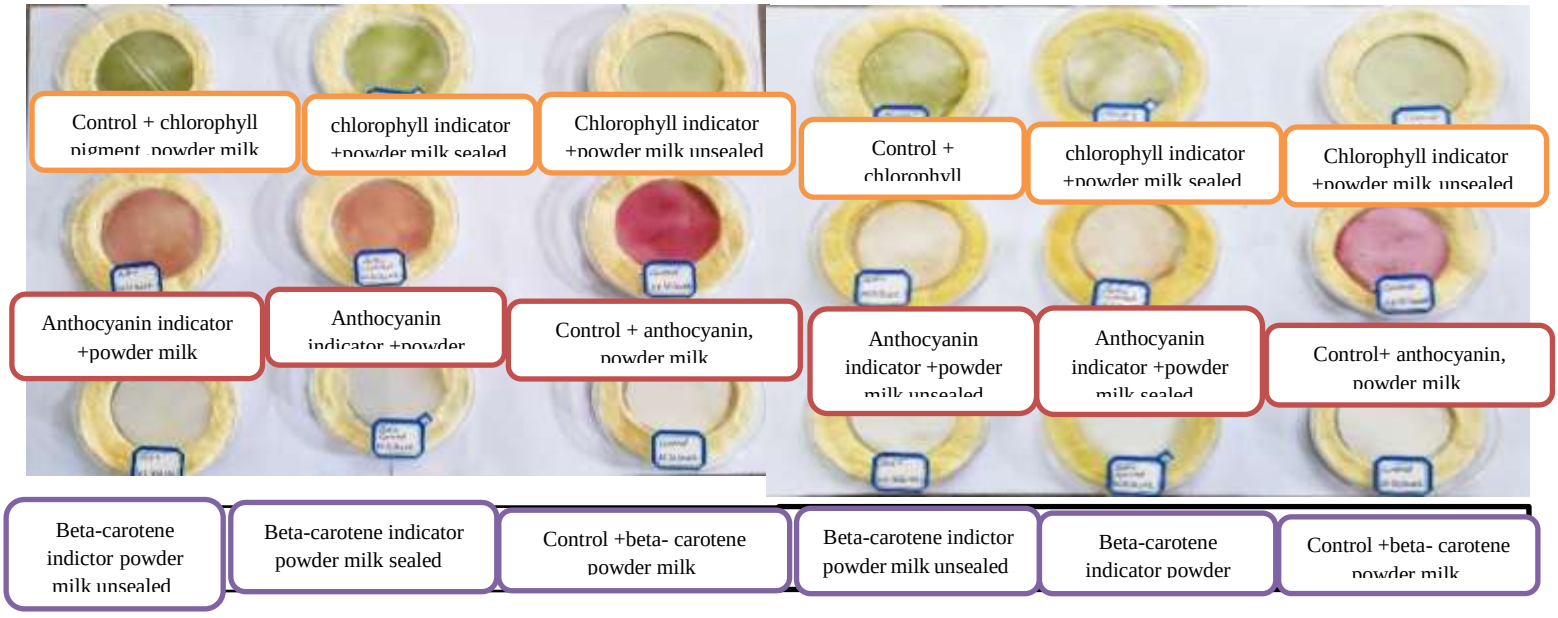

Figure.6 Colorimetric indicators with controls, sealed and unsealed powder milk

The figure.5 contrasts sealed and unsealed powdered milk samples, focusing on pigment degradation as a marker of oxidative damage. Proper sealing effectively limits both moisture and oxygen, the two main determinants of lipid oxidation. The unsealed samples exhibited the sharpest pigment loss, signifying that unmitigated oxygen interaction elevates oxidative stress and that were expressed by alterated color of anthocyanin, chlorophyll and beta carotene indicators to faded colors from red, green and yellow to pale red, yellow and white respectively. Furthermore, the graphs illustrate significant variance between anthocyanin natural dye and samples with the same colorimetric indicators in both sealed and unsealed powder milk states. The highest absorbance level was obtained at 0.620 for red colorimetric pigment, whereas the value at unsealed milk powder sample was 0.082 as consequence of impact oxidation reaction on intensity of anthocyanin colorimetric indicator that is in one hand on the other hand, the producing H2O2 bleached the color as a result of enzymatic reaction in colorimetric indicator was altered the intensity of pigment indicators and effect on reducing absorbance values as a shown in figure.6 after storage .

In contrast, the sealed samples retained pigment stability, underscoring the value of airtight packaging. The control showed negligible pigment change, validating that the milk samples, rather than extraneous variables, caused the variation. Additionally, there are a significant variation among extracted pigments compared to the unsealed and sealed milk powder samples and with increasing duration of storage the colors of colormeteric indicators were changed for fated color as green color of chlorophyll from green to yellow and the absorbance was obtained was 0.235 a half value compared to concentration in green natural extracted dye examining the active colorimetric indicator characteristics reveals that it changes from green to yellow as storage duration and oxidant percentage increase. This change in color can be utilized to estimate how long an oil will last. The color shifts are noticeable to the unaided eye, it should be noted and the somewhat similar to observations of the study by [19]were observed that and used active film, it is observed that with increasing storage time and oxidant percent, the color of the chlorophyll film is changed (from green to yellow), which can be used to estimate the oil expiration time.

Oxidative deterioration in powdered milk begins with unsaturated fatty acids—predominantly linoleic—transforming into hydro peroxides that fragment into smaller aldehydes and ketones [23]. These oxidation products can subsequently destabilize natural pigments such as anthocyanin can be directly oxidized by enzymes such as peroxidase and polyphenol oxidase, resulting in the creation of colorless or brown compounds Moreover, In the presence of enzymes or other oxidative agents, anthocyanins can be rapidly broken down by molecular oxygen and that showed the result in figure 6. The oxidation impact on intensity of pigments and in particular clearly in uncovered samples compared to the controls for all samples and this finding compatible with outcomes mentioned by [24].

This pattern corroborates earlier work indicating that milk fat oxidation is tightly governed by packaging and storage parameters. Even trace ingress of oxygen can set off auto-oxidation in dairy matrices reported that by [7]. Additionally, glucose oxidase-generated hydrogen peroxide emulates the spoilage-related oxidation, permitting a controlled comparison of the sealing approach with natural spoilage in powdered milk.

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Collectively, these findings show that enzymatic pigment systems can work as effective colorimetric indicators for oxidative spoilage in food products like powdered milk and edible oils. The anthocyanin-based indicator label is especially responsive to oxidation markers such as lipid peroxides and reactive oxygen species and breaks down anthocyanin, so this extra efficiency may not be worthwhile. [25] mentioned even in the sample with the lowest ascorbic acid content, the chroma of the encapsulated anthocyanin dropped by 43% or more after processing and only 7 days and that somewhat similar to the results about anthocyanin colorimetric indicator intensity after 1 week of storage in the figure.6 was noted.

Conclusion:

The study's findings showed that a colorimetric approach is a quick, efficient, and optical substitute for the commonly used classical chemical methods for detecting potentially harmful chemical reactions that might take place when fatty foods that are more likely to become rancid and sensitive to oxidation are stored. Additionally, the color shift of the colormeteric indicators allows the customer and marketer to visually identify these reactions, saving the time and effort required for chemical analyses to detect rancidity while also guaranteeing the safety of powdered milk and edible oil products. This capability positions the system for future use in smart packaging and in-field spoilage diagnostics. Furthermore, this study can be expanded by applying it to nuts, and other high-fat foods as chips.

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