FLAVOR Enhancers in Food Processing: Spectrophotometric Determination of Maltol and Ethyl Maltol in Some Commercial Food Samples During Different Storage Conditions

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Abstracts: It is important for flavor additives to maintain adequate thermal stability under high temperature conditions. Since many excellent fragrances are organic and have relatively small molecular weights, they lack thermal stability. As a result, a rapid and accurate method has been developed for simultaneous determination of maltol and ethyl maltol by reaction with iron(III) in sulfuric acid solution. This reaction was the basis for an indirect spectrophotometric method, which followed the development of the pink ferroin product (λmax = 520 nm). It appears that ethyl maltol and maltol follow Beer's law at concentrations of 0.5–20.0 mg/L according to the optimized method. According to the alternative standard method, the LOD values for maltol and ethyl maltol are 0.2 mg/L and 0.3 mg/L, respectively. Commercial food samples were successfully analyzed using the proposed methods. A study was conducted to determine how different storage conditions affected the concentrations of maltol and ethyl maltol. High temperature and sunlight lead to decomposition of these two flavor enhancer concentrations more than 10%.

Keywords: Flavor Enhancer, Maltol, Ethyl Maltol, Spectrophotometric, Storage, Food Samples.

1. INTRODUCTION

In addition to providing valuable information about composition, appearance, texture, flavor, shelf-life, safety, processability, and microstructure, food analysis also ensures the quality of the product and concentration of food contaminants [1-4]. Thus, in food industry processes, food additives play a crucial role in maintaining or improving food nutritional value, taste, freshness, color, and safety [5, 6]. Maltol (MA) and ethylmaltol (EMA) are important flavor enhancers in food. In the food industry, maltol (3-hydroxy-2-methyl-4-pyrone) has been marketed as a flavor enhancer. In heated-processed ginseng, MA is one of the products of the Maillard reaction between maltose and amino acids. As a natural antioxidant, food preservative, and flavor potentiator, MA is widely found in nature. Meanwhile, foods that have been heat-processed often contain MA as one of the products of the Maillard reaction [7]. Maltol's synthetic homologue, EMA, has been available since 1967 and is approximately six times more effective than MA [8]. Commercial foods like beverages, chocolates, cookies, and candies, use these compounds to enhance their fragrance [9]. Even though these compounds can enhance the scent of foods, they are synthetic perfumes and food additives. They can cause vomiting, nausea, headaches, and could affect liver and kidneys function if consumed in large amounts [10]. Therefore, it is important to determine the amount of these chemicals in food.

Foods are exposed to different conditions in terms of temperature and light effect especially sunlight which leads to different chemical and physical changes. It is possible that these factors affect the foods quality as a result of the wrong storage in large warehouses and markets [11]. MA and EMA are determined by analytical methods including atomic absorption spectrometry [12], high performance liquid chromatographic method [13-15], gas chromatography-mass spectrometric method [16], UV-Visible spectrophotometric methods [17-19], chemiluminescence [20], liquid chromatography-mass spectrometry [21], and electrochemical methods [22-24]. Because of its intrinsic simplicity, inexpensive, and widespread availability in quality control laboratories, spectrophotometric method is perhaps the most practical analytical technique for routine examination [25-27].

Maltol, ethyl maltol, vanillin and ethyl vanillin are food additives, and they have well defined UV spectra. However, these overlapped seriously, and it is difficult to determine them individually from their mixtures without a

pre-separation. In this paper, chemometric approaches were applied to resolve the overlapping spectra and to determine these compounds simultaneously. The analysis of these four compounds was facilitated using an orthogonal array data set consisting of absorption spectra in the 200–350 nm ranges obtained from a calibration set of mixtures containing these compounds. With this dataset, seven different chemometric models were built, such as classical least squares (CLS), principal components regression (PCR), partial least squares (PLS), and artificial neural networks (ANN). These chemometric models were then tested using a validation dataset constructed from synthetic solutions of these four compounds. The analytical performance of these chemo metric methods was characterized by relative prediction errors (RPE) and recoveries. The proposed methods were successfully applied to the analysis of commercial food samples. It was found that the radial basis function artificial neural networks (RBF-ANN) gave better results than other chemometric methods. PLS, PCR, DPLS, and DPCR also give satisfactory results, while CLS and DCLS perform poorer. It was also found that there was no advantage to pre-treating spectra by taking derivatives. The four compounds, when taken individually, behaved linearly in the 1.0–20.0 mg/L concentration range, and the limits of detection for MA, EMA, VAN and EVA were 0.39, 0.56, 0.49 and 0.38 mg/L , respectively [17]. A method based on isotope dilution headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry was developed for the simultaneous determination of MA and EMA. Optimal values of relevant parameters affecting extraction efficiency and detection sensitivity were determined and then applied to the analysis of real samples. With the advantages of good reliability and the capacity for simultaneous detection, the method can be applied to detect adulteration and evaluate any health risks in a range of foods by the four flavor compounds [16].

In this work, a simple, fast, low-cost, and sensitive spectrophotometric method has been developed for the determination of two analytes, maltol and ethyl maltol. The method depends on the oxidation of maltol and/or ethyl maltol with iron (III) in a sulfuric acid medium, and then reaction of formed Fe(II) with o-phenanthroline (o-Phen). The calibration graphs were constructed. The method was verified, compared, and applied to the real food samples. Maltol and ethyl maltol concentrations were studied under different storage conditions.

2. MATERIEL AND METHODS

2.1. Instrumentation

The pH measurements were conducted with an Orion EA940 pH-meter that was calibrated before use, with absolute accuracy limitations for the pH readings being specified by NIST buffers. The spectrophotometer used was a JENWAY 6405 UV/Visible spectrophotometer with a 1.0 cm glass cell. The temperature of the solutions and samples was set as needed for tests using a handmade heated water bath.

2.2. Chemicals and Reagents

Analytical grade chemicals and deionized water were used to prepare all solutions. In a 100 mL volumetric flask, suitable weight aliquots of maltol (1.0 g/L) and ethyl maltol (1.0 g/L) stock solutions were prepared; they were dissolved in deionized water and diluted to the desired concentration. An o-phenanthroline solution (0.02 mol/L), 0.04 mol/L Fe(III) solution, and 1.0 mol/L sulfuric acid solution were prepared by taking suitable weight (or volume) of the reagents and dissolving them in deionized water.

2.3. General Procedure

A volume of 2.0 mL of 1.0 mol/L of Fe(III) and 0.03 mL of 1.0 mol/L of sulfuric acid was placed in a 10 mL volumetric flask. Then, 1.0 mL of MA was added, and the flask was allowed to stand for 2.0 min (or 5.0 min for EMA) in a temperature-controlled water bath at 25 ℃. Then, 2.0 mL of 3.0 mol/L o-Phen was added and the volume was completed with deionized water. Finally, the absorbance was recorded after 1 min at a maximum wavelength of 520 nm. By plotting absorbance versus concentration of the analyte during a fixed-time interval (optimum), a calibration graph was created.

2.4. Maltol and Ethyl Maltol Determination in Food Samples

Several commercial food samples were purchased from the market in Erbil City, Iraq. 100 grams of solid food samples, such as biscuits and jelly, were ground to a fine powder. While 100 mL of the liquid food sample, such as beverage and julep, was filtered. 75 grams of the powder or 75 mL of the filtrate were then placed into a 250 mL Erlenmeyer flask (with a screw cap) and shaken for 150 minutes. The mixture was then transferred to a 10 mL centrifuge tube and centrifuged for 10 minutes at 4000 rpm. Analyses were conducted on the clear portion of the mixture in the tube. 5.0 mL of chloroform and an appropriate amount of this sample were added to a flask with a volume of 25 mL. After dilution with water, they were thoroughly mixed [18]. Using the general procedure described above in Section 2.3, such a solution was used for analysis. Figure 1 illustrates the steps involved in determining MA and EMA.

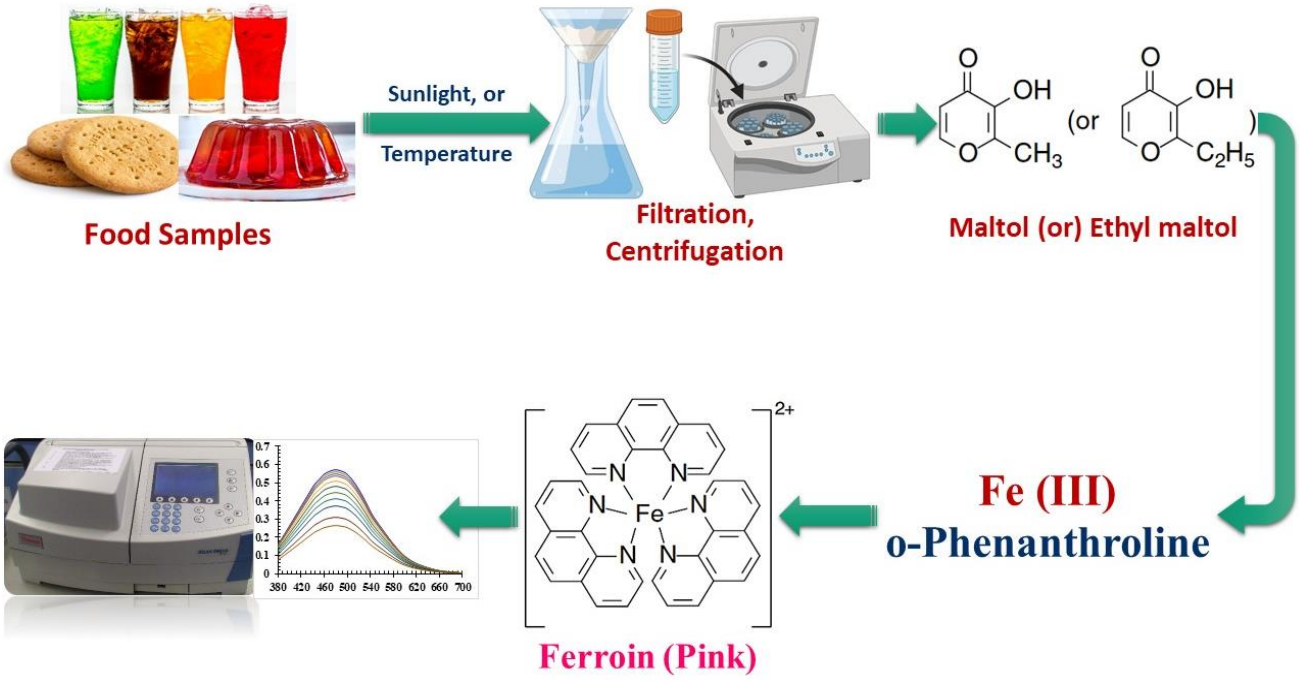

Figure 1. Illustration of maltol and ethyl maltol determination steps.

3. RESULTS AND DISCUSSION

3.1. Absorption Spectra

According to previous studies [18, 28], the pink ferroin complex is formed by the reduction of Fe(III) by MA or EMA in the presence of o-Phen in sulfuric acid as shown in Figure 2. A difference has been observed between the oxidation rates of MA and EMA with Fe(III) in preliminary investigations. It involves two processes: the rapid reduction of iron (III) by MA and the relatively slow reduction of iron (III) by EMA, followed by complex formation of iron(II) with o-Phen [18]. For this reason, separating MA and EMA is best accomplished by varying reaction rates.

Figure 3**Figure** shows a spectrum of colored ferroin obtained from experiments under experimental conditions between 400 and 800 nm at a reaction time of 2.0 min. As a result of the formation of colored ferroins, the absorption spectrum shows a maximum band at 520 nm.

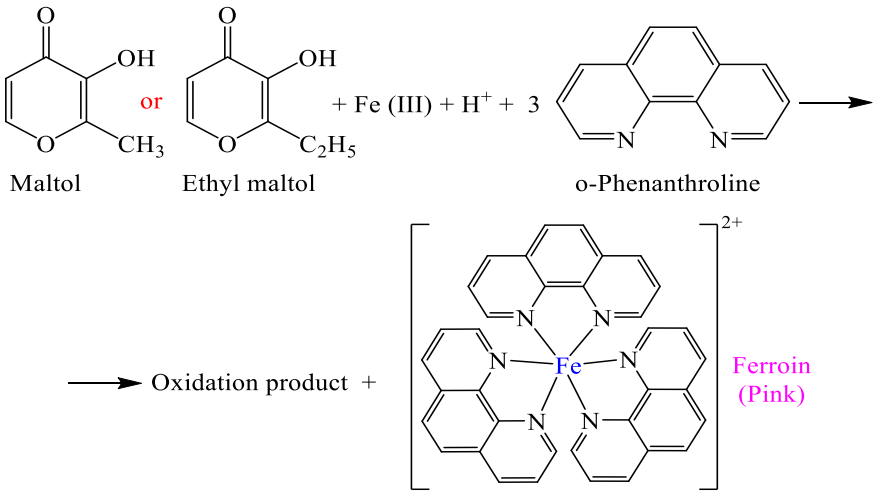

Figure 2. Redox reaction of MA or EMA by Fe(III) in the presence of o-Phen in acidic medium.

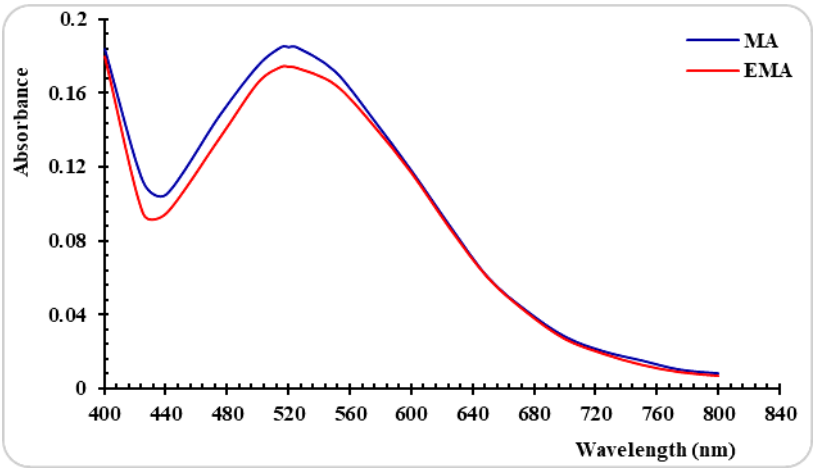

Figure 3. Absorption spectra of MA (20 mg/L) and EMA (20 mg/L) using 0.002 mol/L of Fe(III), 0.0003 mol/L o-Phen, 0.01 mol/L H_2SO_4, at reaction time 2.0 min at 25 ℃.

3.2. The Reaction Conditions Optimization and Calibration

The effects of Fe(III), o-Phen, acid type and concentration, reaction time, and temperature on MA and EMA determination were optimized. The results showed that the maximum absorbance's obtained with 0.002 mol/L of Fe(III), 0.0003 mol/L of o-Phen, 0.016 mol/L of sulfuric acid, and reaction time 2.0 min, and 4.0 min for MA and EMA at 30 ℃, respectively as shown in Figure 4**Figure** and Figure 5**Figure**a, and b. Under the optimized conditions, rectilinear calibration graphs were obtained in the concentration ranges from 0.5 to 20.0 mg/L for MA and EMA (Figure 5**Figure**c). The comparison of the proposed method with previous published methods according to the limits of detection, limits of quantifications, and linear calibration range are given in Table 1**Table**.

To evaluate the precision of the method, measurements were performed under conditions of repeatability and reproducibility. It was checked for the error attributable to sample handling and preparation and instrument response for a standard solution of the analyts. The precision of the method was determined, under the optimal working conditions, by five absorbance measurements of three concentration levels (low, medium, high) for each analyte. In addition, the recovery rates of pure MA and EMA at three concentration levels encompassing the high, medium, and low ranges of the calibration graphs were used to determine the method's accuracy (Table 2**Table**).

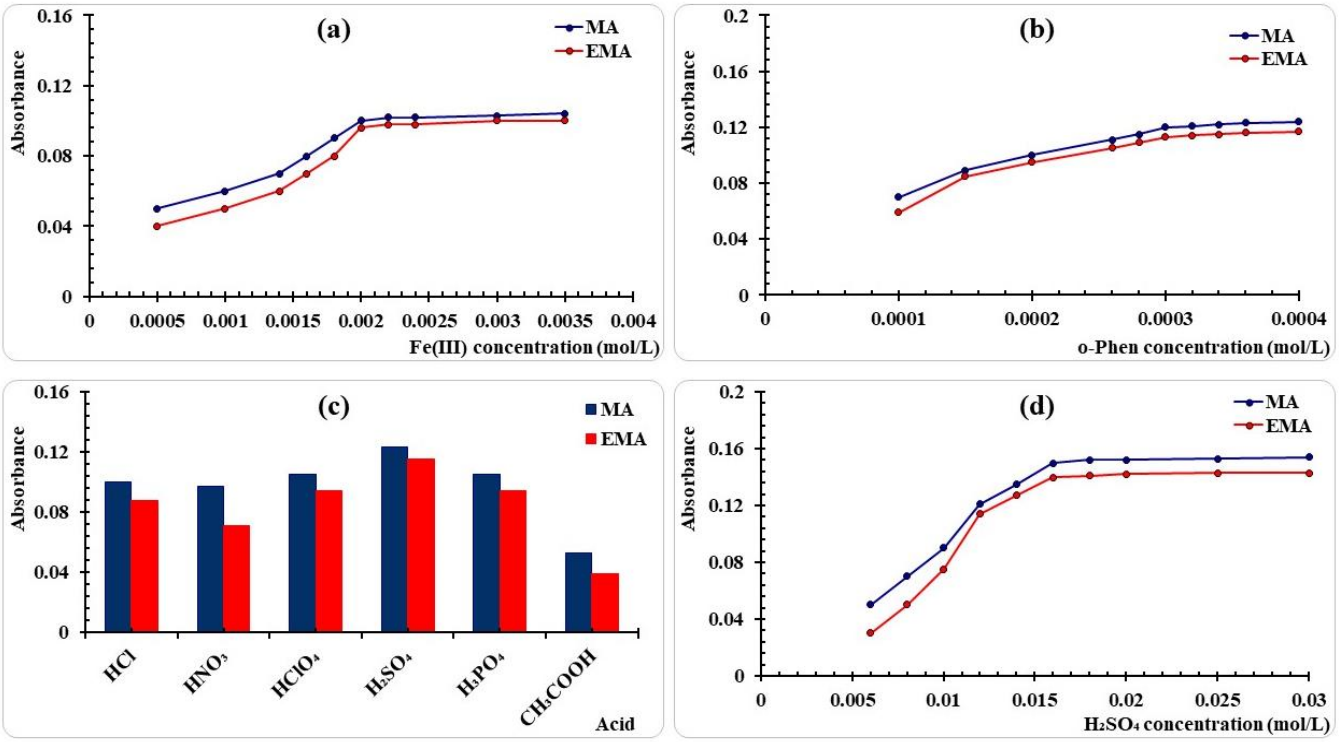

Figure 4. optimization of experimental parameters, (a) Fe(III) concentration; (b) o-Phen concentration; (c) acid type; and (d) sulfuric acid concentration.

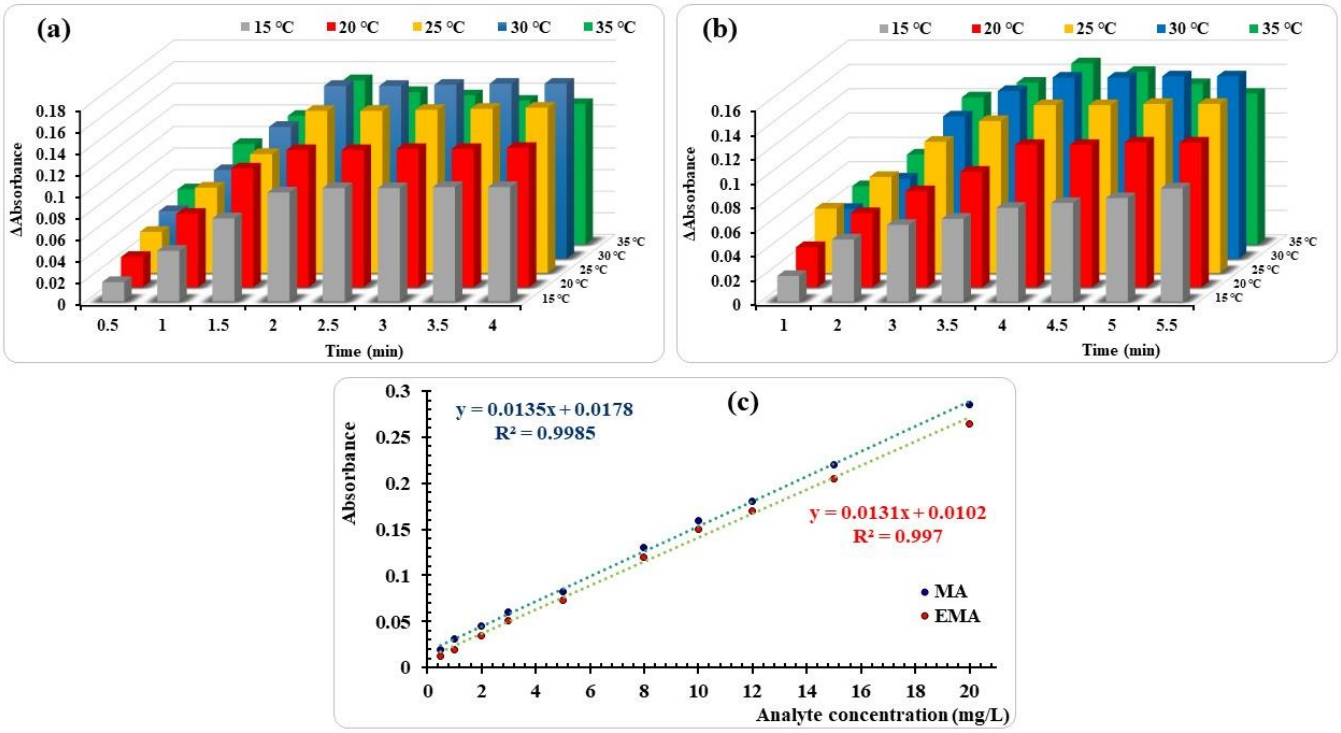

Figure 5. Effect of time and temperature on the determination of (a) MA; and (b) EMA; and (c) calibration graph using the proposed method.

Table 1. Comparison of the linear range and limit of detection (LOD) of the proposed method with some reported methods.

Method	Analyte	LOD (mg/L)	Linear range (mg/L)	Reference
Chemiluminescence	MA	10.0	0.50 – 4.00	[20]
Spectrophotometry	MA EMA	0.39 0.56	1.00 – 20.00 1.00 – 20.00	[17]
Spectrophotometry	MA EMA	1.60 1.40	4.00 – 76.00 4.00 – 76.00	[18]
Spectrophotometry	MA EMA	0.20 0.30	0.50 – 20.00 0.50 – 20.00	This method

Table 2. Evaluation of the accuracy and precision of the proposed spectrophotometric method.

Analyte	Analyte concentration (mg/L)		Recovery% ± SD
	Added	Found*	
MA	5.00	4.93	98.60 ± 0.93
	10.00	9.91	99.10 ± 0.82
	15.00	14.95	99.67 ± 0.24
EMA	5.00	4.87	97.40 ± 1.07
	10.00	9.88	98.80 ± 0.91
	15.00	14.92	99.47 ± 0.37

* Average value of five determinations.

3.3. Selectivity of the Method

The impact of various substances on the analysis of a combination of MA (10.0 mg/L) and EMA (10.0 mg/L) was examined under ideal reaction conditions in order to study the selectivity of the proposed approach. The concentration of additional species that results in less than a ±5.0% recovery percentage was the tolerance limit. The obtained results indicated that aspartame, fructose, citric acid, glucose, sucrose, Ca, K, and Na ions did not interfere at a 200:1 interferent/anlyte concentration ratio; synthetic colorants that used in food industry, like indigo carmine, brilliant blue, ponceau 4R, amaranth, tartrazine, and sunset yellow produced interferences at about 100-fold concentration level relative to that of the flavor enhancers (Figure 6**Figure**). Fe (II), Cu (II), Mn (II), Zn (II), ethyl vanillin, and vanillin produced only small effects even at a 40:1 interferent/analyte ratio. Significant interference with the decision was caused by ascorbic acid. Thus, it is crucial to extract the analytes from food samples using chloroform [18]. Thus, these interferences are reduced.

3.4. Determination of Maltol and Ethyl Maltol in Food Samples

This method was applied to several food samples available in local markets for the determination of MA and EMA using verified calibration. As shown in Table 3TABLE, there is good agreement between the results obtained from the proposed method and those produced by the reference UV-spectrophotometric approach. The percentage of recoveries is uniformly consistent. This shows that the suggested method can yield acceptable results for the measurement of MA and EMA in real food samples.

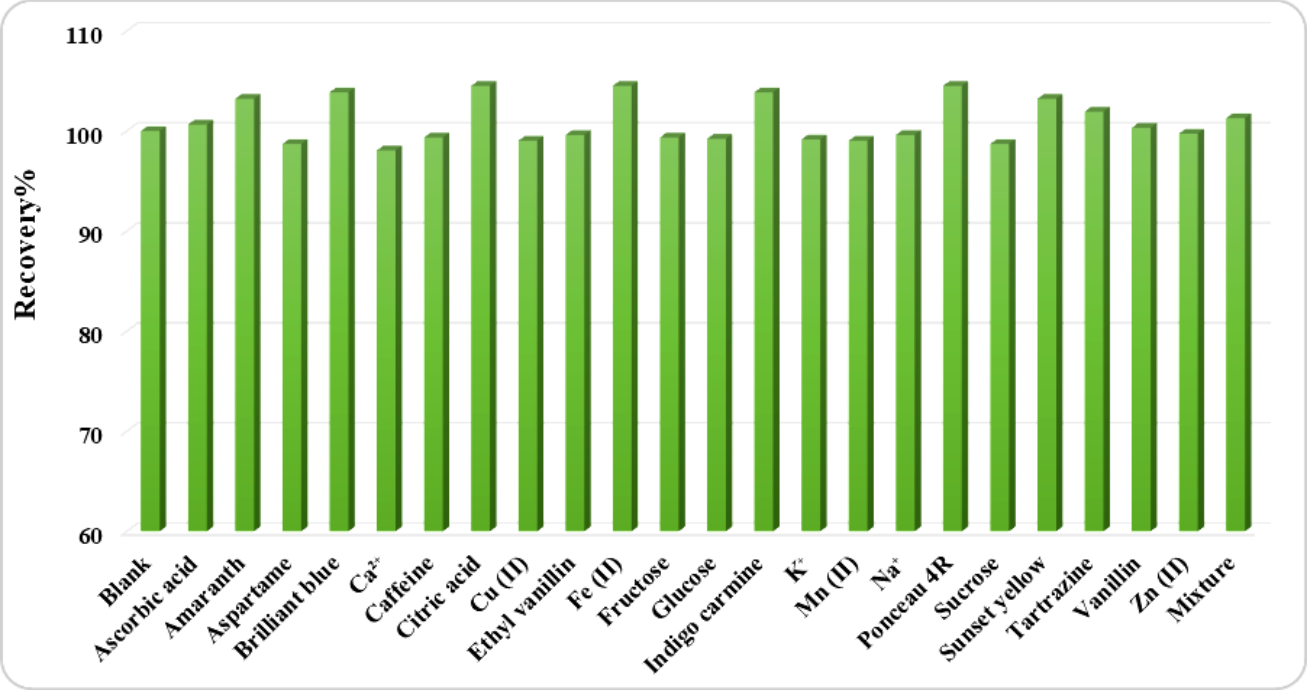

Figure 6. Effect of interfering compounds on the determination of MA and EMA using the proposed method.

Table 3. Application of the proposed method for the determination of MA and EMA in real food samples.

Samples	Proposed method (µg/g)		Reference method (µg/g)		Recovery% ± RSD*	
	MA	EMA	MA	EMA	MA	EMA
Jelly 1	10.25	N.D[a]	10.11	N.D.	101.38	0.00
Jelly 2	9.78	N.D.	9.54	N.D.	102.52	0.00
Biscuit	23.91	N.D.	24.76	N.D.	96.57	0.00
Beverage 1	0.00	23.84	N.D.	24.20	N.D.	101.51
Beverage 2	0.00	19.22	N.D.	19.14	N.D.	99.58
Julep	16.64	N.D.	16.38	0.00	101.59	0.00

* Recovery%= (Found by proposed method/Found by reference method)×100; RSD= Relative Standard Deviation; [a]ND = Not detected.

4. STORAGE EFFECTS ON FLAVOR ENHANCERS

In some applications flavor additives should maintain adequate thermal stability in high temperature conditions. However, most of the excellent fragrances lack thermal stability due to their relatively small molecular weight and pure organic structure. For example, the volatilization temperatures of MA and EMA are in the range of 90 − 170 ℃, so they cannot stably exist in high temperatures [29].

Evaluation of the influence of storage on the concentration of the two studied flavor enhancers (MA and EMA) in real food samples is one of the study's top goals. Since these food samples are subjected to a variety of temperatures and sunlight effects, the amount of MA and EMA in these samples' changes with time. Because of improper storage in sizable warehouses and markets, these variables have an impact on the foodstuffs in such locations. This may occur as a result of keeping foodstuffs in locations that are directly exposed to sunlight, which in turn causes physical and chemical changes in these foods. Because of a lack of sufficient ventilation and cooling for the storage conditions, temperatures in certain warehouses can reach 60 °C or more in the summer. Some of these foods include colored components that can be damaged by the effects of temperature, UV, and sunlight, resulting in a loss in product quality. In order to perform this study, samples of foods were collected and split into two groups.

MA and EMA concentrations were determined at periods after the first group was exposed to various temperatures and the second group to direct sunlight.

4.1. Effect of Temperature

In the first group of solutions, different temperatures were maintained in a dark place during storage without exposure to light. A five-month study was conducted on the effect of storage temperatures at 7, 25, 35, 45, and 55 ℃. MA and EMA concentrations were calculated on the 15th day of storage, and after each month for five months.

Both Figure 7**Figure** and Figure 8**Figure** illustrate how storage temperature and storage period affect MA and EMA concentrations, respectively. Five months after the experiment, the concentrations of both analytes in the standard solution and food samples are almost constant and do not change except at very small rates at low temperatures (7 ℃, refrigerator) and room temperatures (25 ℃). Due to the decomposition of MA and EMA, analyte concentrations decrease with an increase in temperature from 35 to 55 °C with increasing storage time [29]. The 10.00 mg/L standard maltol solution concentration changed to 9.87 mg/L at 35 ℃, 9.77 mg/L at 45 ℃, and 9.56 mg/L at 55 ℃ after five months. While standard EMA concentration changed from 10 mg/L to 9.84 mg/L at 35 ℃, 9.58 mg/L at 45 ℃, and 8.90 mg/L at 55 ℃ after five months. In addition, both flavor enhancer concentrations are decreased about 10% after five months storage in dark place at 55 ℃. Additionally, because the storage conditions are in a dark place, the heat effect is almost identical for standard solutions, beverages, jelly, julep, and biscuits.

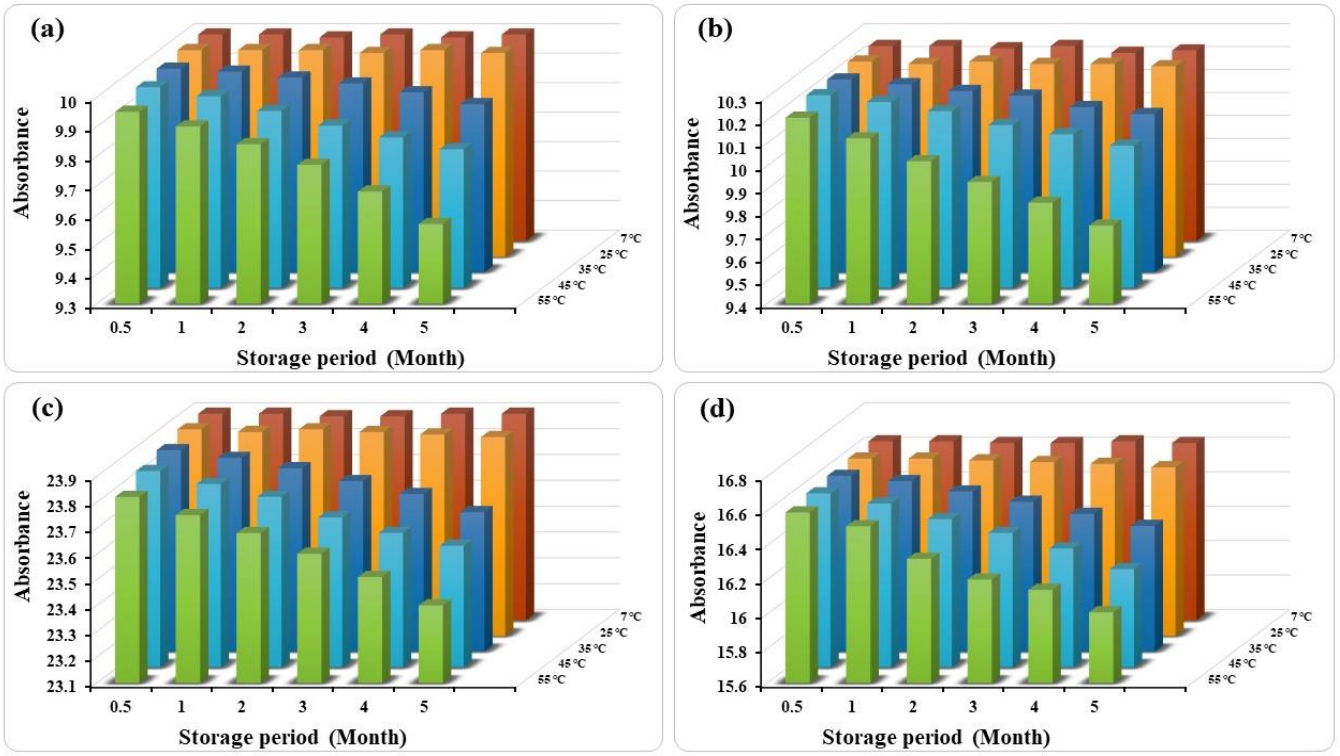

Figure 7. Storage temperature effect on the maltol concentration in (a) standard solution, (b) jelly, (c) biscuit, and julep sample.

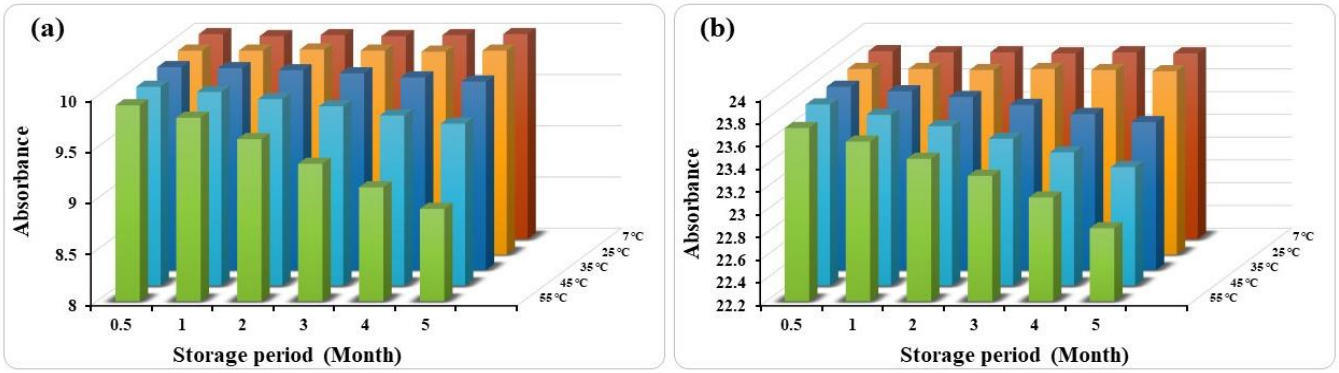

Figure 8. Storage temperature effect on the ethyl maltol concentration in (a) standard solution, and (b) beverage sample.

4.2. Effect of Sunlight

As foods are stored in some stores in open areas that are exposed to sunlight, or near glass windows that are directly exposed to the sunlight. In other stores, these foodstuffs are placed in refrigerators to keep them cool, but these refrigerators are exposed to the sunlight. To address this issue, the second group of food samples that contain MA and/or EMA were exposed to sunlight for specific periods during May, June, July, and August. In each case, analyte concentrations were determined.

Sunlight's effect on MA and EMA concentrations is shown in Figure 9**Figure**. In the standard solution of the two flavor enhancers, the concentration decreases more slowly than in the real food samples. Furthermore, sunlight and ultraviolet rays may lead to the degradation of MA and EMA and formation of harmful chemicals. According to the figure, MA and EMA concentrations decrease faster in the months of July and August due to the intense sunlight and high temperatures in these months. Sunlight with heat generally decreases the concentration of flavor enhancers in stored foods more than just high temperatures alone.

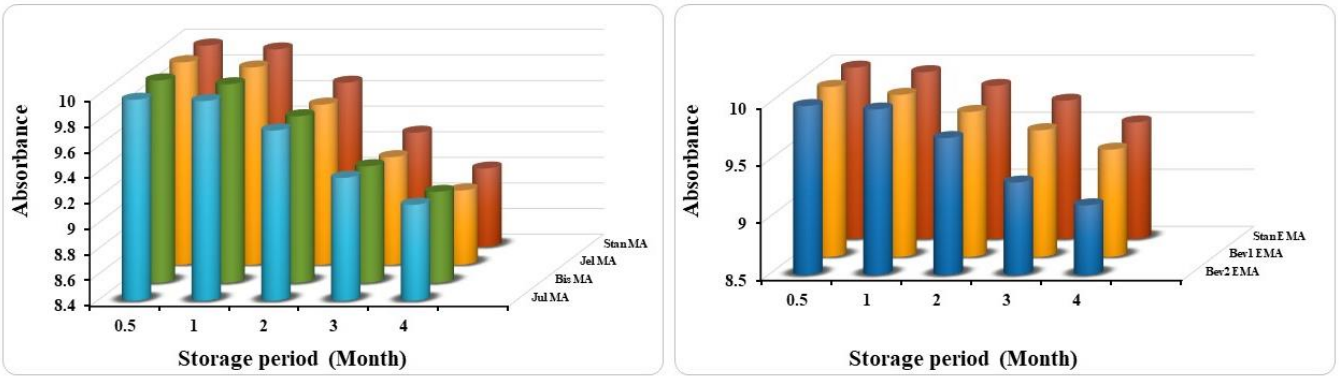

Figure 9. Effect of sunlight on the concentration of (a) maltol, and (b) ethyl maltol.

CONCLUSION

A simple, fast, and cheap spectrophotometric method has been researched and developed for the simultaneous determination of the common food flavor enhancing compounds, maltol, and its synthetic homologue, ethyl maltol. The significance of this method is that it enables a simple quantitative discrimination of the two compounds. This is important since biological health and safety concerns have been raised about ethyl maltol. The suggested method utilizes the multivariate kinetic approach because the UV–visible spectra of the two compounds are almost the same. An indirect method, which follows the development of the pink color of the ferroin reagent in the reaction with the two analytes, facilitates the discrimination of the compounds on a reaction time basis. As a result of this study,

storing of foods at varying temperatures and exposing them to sunlight for various periods of time, MA and EMA degradation into potentially harmful compounds increased as temperature and exposure to sunlight increased.

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