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Catalase Activity in Hot-Air Dried Mango as an Indicator of Heat Exposure for Rapid Detection of Heat Stress

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Abstract: The growing market for dried fruits requires more attention to quality parameters. Mango and other tropical fruits are commonly dried at temperatures ranging from 40 °C to 80 °C. Convincing evidence suggests that the nutritional quality of dried fruits is best preserved when dried at low temperatures ≤50 °C, whereas increasing drying temperatures lead to the degradation of the most valuable nutrients inside the fruit. Currently, there is no system or direct measurement method that can assist in identifying the quality deterioration of dried fruits caused by excessive heat exposure during drying. From this perspective, the activity of the heat-sensitive enzyme 'catalase' was used for the first time to evaluate and compare mango slices dried at 40 °C, 60 °C and 80 °C. Various methods, including direct and indirect flotation tests and spectrophotometric measurements, were explored to measure the residual catalase activity in the dried samples. Results showed that the spectrophotometry and indirect flotation test produced the best results, revealing a significant difference ($p < 0.05$) in the catalase activity of mango slices dried at 40 °C, 60 °C and 80 °C, which the direct-dried mango flotation test failed to predict. Furthermore, this study demonstrates the potential applicability of catalase activity to indicate heat stress in dried mango slices processed at different temperatures.

Keywords: fruit nutrients; degradation; convective drying; catalase activity; flotation time; spectrophotometry; Sindri

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1. Introduction

The consumption of dried fruits as a healthy snack has increased tremendously in recent years, mainly because of their high nutritional value [1]. This change in consumer behavior led to a rise in dried fruit production, which increased by about 29.74% from 2.2 million metric tons to 2.9 million metric tons between 2009 and 2021 [2]. To meet the increasing demand for dried fruits, fruit drying industries are focusing more on their bulk production without paying much attention to the preservation of nutrients and drying conditions. Generally, fruits are dried by various methods such as convective hot-air drying, freeze-drying, vacuum drying, microwave drying and infrared drying. Among these drying methods, hot-air drying is the most commonly used due to its easy operation, low investment and operating costs. However, this process leads to a loss of valuable nutrients within the fruits. Many studies have successfully proven that the nutritional quality of dried fruits is directly related to the fruit material and the operational drying conditions, especially the temperature. Drying at high temperatures of ≥80 °C causes several degradation reactions that not only affect sensory attributes (color, texture and flavor) but also participate in the destruction of most valuable nutrients (vitamins, enzymes, carotenoids, antioxidants and other bioactive components or phytochemicals) within the fruit [1,3–5]. However, a low drying temperature, such as 40 °C, helps to preserve the

natural components of the fruit [6–8]. Unfortunately, there are no strict regulations for the drying of fruits, and companies are free to choose the drying temperature that best suits their needs. Most often, fruits are dried at high temperatures to reduce drying time and maximize dryer capacity. At the same time, sales departments are well aware that visual preferences, especially color, have always been an important concern for consumers. Many researchers have also documented that color plays a key role in the acceptance or rejection of a product even without tasting [9,10]. In modern research, many pretreatment techniques have been successfully developed that can help preserve the color of dried fruits [11,12]. Therefore, selecting a product based on color can mislead consumers and may not meet their nutritional quality requirements. Nowadays, consumers are becoming more concerned about their diet and the nutritional value of food. Therefore, it is crucial to identify new quality parameters that can predict the quality degradation of dried fruits due to excessive heat exposure and to assure consumers that the dried fruits they consume are rich in health-promoting nutrients.

Among the dried fruits, mango is very popular and is usually dried at temperatures ranging from 40 °C to 80 °C, to a target moisture content of 10–15 g 100 g^{-1} wet basis [13,14]. Several researchers have extensively documented the effects of drying temperatures on quality attributes such as texture, color, total soluble solids, sugars, fiber, moisture content, vitamins, minerals, antioxidants, volatile compounds, carotenoids, phenolic content and phytochemicals of dried mangoes [3,4,11,12,15–20]. In most of these studies, it was well observed that the nutritional quality of dried mango slices was best preserved at drying temperatures from 40 °C to 60 °C, while at higher drying temperatures, the nutritional and health benefits of dried mango slices were strongly compromised due to the loss of heat-sensitive nutrients, particularly antioxidants, volatile compounds, phenolic contents, enzymes, carotenoids (luteoxanthin, violaxanthin and neoxanthin) and phytochemicals. Although much information about the influence of drying temperatures on mango nutritional quality parameters is readily available in the literature, researchers widely agree that low drying temperatures facilitate the retention of most valuable nutrients within the dried mangoes. However, the major challenge is that after drying, it is hard to determine whether the dried samples were treated at low or high temperatures. To date, there has been no established protocol or direct measurement method that can distinguish the difference between mangoes dried at different temperatures. Instead of testing a variety of quality parameters, a simple measurement of heat stress of dried mango slices would be desirable as a preliminary screening test to predict quality degradation during drying. Therefore, in this study, the activity of the heat-sensitive enzyme 'catalase' was used for the first time as an indicator of temperature exposure during the drying of mangoes. In agreement with our previous work, Mukhtar et al. [7] found that where catalase was found as a heat-sensitive enzyme in mango, a significant effect on its activity was observed at drying temperatures 40 °C to 80 °C. This work aimed to develop a simple, rapid and appropriate method that can help to differentiate the dried mango slices of 40 °C, 60 °C and 80 °C based on their residual catalase activity. Various methods, including direct and indirect flotation and spectrophotometric measurements, were analyzed and compared to achieve the desired objective.

2. Materials and Methods

2.1. Materials

Fresh, ripe mango (*Mangifera indica* L.) of the variety Sindri imported from Punjab, Pakistan, was purchased from a local fruit seller in Stuttgart, Germany. The fruits had an average weight of 327.49 ± 16.58 g. The total soluble solids of 17.58 ± 1.61° Brix were measured using a digital refractometer (ATAGO PR -201 palette, ATAGO Co. Ltd., Tokyo, Japan), while the average initial moisture content of 85.04 ± 1.83% w.b. was determined by drying in an oven at 105 °C for 24 h [21], and the water activity of 0.92 ± 0.02 was analyzed using a ventilated hygrometer system (Rotronic A2, Rotronic AG, Basserdorf, Switzerland) after 30 min in a thermostatic cell at 23 °C.

2.2. Drying Process

Mangoes were washed, peeled manually with a knife and cut into slices (4 cm × 2 cm × 0.8 cm) using an electric food dicer (MultiSchneider Serano 7, Ritter, Groebenzell, Germany). The drying experiments were conducted using a high-precision laboratory dryer designed at the Institute of Agricultural Engineering, University of Hohenheim, Stuttgart, Germany [22]. The temperature, humidity and air velocity inside the drying chamber were monitored using PLC software. The temperature of the air was measured with Pt-100 sensors at different locations (accuracy ± 0.01 °C). The airflow was measured by differential pressure monitoring sensors (orifice meter) at an accuracy of ± 0.05 m s^{-1}. The humidity was adjusted by changing the dew point temperature using a psychometric chart. All experiments were performed in duplicates by using the over-flow chamber of the dryer at temperatures of 40 °C, 60 °C and 80 °C, maintaining a constant specific humidity of 10 g kg^{-1} and an air velocity of 1.4 m s^{-1}. The samples were dried until the final moisture content of the mango slices reached a level of approximately 11% w.b. This moisture content is under the hygienically safe water activity ≤0.6 [23,24]. After drying, the mango slices were cooled, packed airtight in polyethylene bags and stored at room temperature before use.

2.3. Colorimetric Measurement

Color parameters of fresh and dried mangoes were measured with a colorimeter (model CR-400, Minolta Co., Ltd., Osaka, Japan) using the CIE *L*a*b** color system. The parameter *L** represents the brightness of the color, *a** the color range (green to red), and *b** the color range (blue to yellow). Before the measurement, the instrument was calibrated with a standard white plate D65 (Y = 87.5, x = 0.3180 and y = 0.3355). Twenty measurements were taken separately for each experiment by placing the colorimeter head directly above the slices. The hue angle was calculated by using the equation described by Nagle et al. [25]:

$$h^o = \frac{180 \times Arc\ tan\left(\frac{b*}{a*}\right)}{\pi}, \quad (1)$$

where, h° is the hue angle; *a** and *b** are the color values in CIE *L*a*b** color space.

2.4. Quality Prediction Test

In the quality prediction test, the activity of the heat-sensitive enzyme 'catalase' was used as a new approach to differentiate between the mango slices dried at 40 °C, 60 °C and 80 °C. To observe the catalase activity in the dried samples, various methods such as the flotation test and spectrophotometric measurements were performed.

2.4.1. Flotation Test

The flotation test is one of the simpler and more straightforward methods to observe the catalase activity and is based on the gas formation when catalase is reacting with hydrogen peroxide (H_2O_2). This test was carried out as described by Chan et al. [26] with some modifications.

Direct Flotation Test

The direct flotation test is a rapid assessment method performed directly on dried mangoes. The underlying principle of this approach is that dried mango with more catalase enzymes can react faster with hydrogen peroxide (H_2O_2) solution and rise to the top of the solution in less time by generating more O_2 bubbles on the fruit surface. The color of mango slices dried at 40 °C, 60 °C and 80 °C seemed closer to each other, were selected at random and kept separately at room temperature. The slices were cut into small quadratic cuboids (10 × 10 mm), as shown in Figure 1.

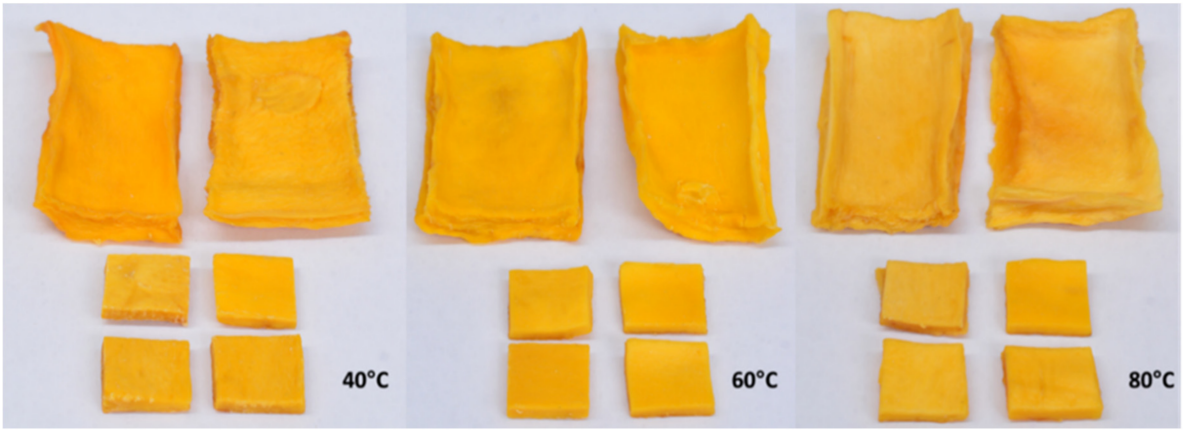

Figure 1. Dried mango slices and cuboids (10 × 10 mm) of 40 °C, 60 °C and 80 °C.

Three 200 mL beakers containing 130 mM hydrogen peroxide (H_2O_2) substrate (pH 7.0) were placed at a height of 50 mm on the heating plates set at 25 °C. Twenty cuboids from different mango slices dried at 40 °C, 60 °C and 80 °C were tested separately by placing them at the bottom of the beaker with clean forceps. The solution started bubbling on the fruit surface due to the reaction between H_2O_2 and catalase. The dried samples gradually floated up to the surface of the H_2O_2 solution. The rising time of the samples was recorded once they reached the surface of the solution. The rate of enzyme activity was calculated using the following equation:

$$k = \left(\frac{s}{t} \right), \tag{2}$$

where k is the rate of enzyme activity (mm s^{-1}), s is the distance (mm) and t is the rising time of the mango pieces (s). The influence of density on the rising time was determined by randomly selecting 10 cuboids from the mango slices dried at 40 °C, 60 °C and 80 °C. The density was calculated as described by Mitrevski et al. [27]:

$$\rho = \left(\frac{m}{V} \right), \tag{3}$$

where m is the mass of the sample (g) and V is the volume (cm^3). The volume was measured using a pycnometer (BLAUBRAND, Wertheim, Germany) with an inner volume of 25 mL. The methodology was conducted based on Yan et al. [28]. Toluene was used as a non-water-soluble liquid. At 22 °C ambient temperature, the density of toluene was noted as 0.862 g cm^{-3}. The volume of the samples was calculated as:

$$V = V_f - \left(\frac{m_{f+s+tol.} - m_f - m}{\rho_{tol.}} \right), \tag{4}$$

where V_f is the volume of the weighing glass (cm^3), $m_{f+s+tol.}$ is the mass of the weighing glass, sample and toluene (g), m_f is the mass of the weighing glass (g), m is the mass of the sample (g) and $\rho_{tol.}$ is the density of toluene (g cm^{-3}).

In addition, the surface changes of dried mangoes were characterized using scanning electron microscopy (JSM-IT100, JEOL GmbH, Freising, Germany). The samples of approximately 3 mm × 3 mm obtained from the central zone of the dried mango slices were fixed on a gold-plated cylinder and placed on the sample platform. Several images were captured from different surface areas of the samples at magnifications of ×50–250 under high vacuum conditions with an accelerating voltage of 2.5 kV.

Indirect Flotation Test

For the indirect flotation test, filter discs soaked with catalase extracts were used instead of directly dried mango to exclude the influence of density, air entrapment and

surface changes of dried mango on the flotation time. Willits & Babel [29] introduced the disc flotation test to measure catalase activity in milk. Ten catalase extractions were made from fresh mangoes and mangoes dried at 40 °C, 60 °C and 80 °C as described by Mukhtar et al. [7]. To prepare each extraction of fresh mango, the flesh of six mangoes was cut into small pieces and homogenized using a vortex mixer (Polytron 2500E, Kinematica, Luzern, Switzerland). In total, 10 g of the resulting homogenate were transferred into each falcon tube, and 10 mL of extraction buffer (0.1 M Potassium phosphate buffer, pH 7.0) was added. After mixing, the samples were further centrifuged (Z326K, Hermle Labortechnik GmbH, Wehingen, Germany) at 13,400 rpm for 10 min at 4 °C. The supernatants were collected and kept separately at room temperature for further analysis. For each extraction of dried mango, different dried slices from the same treatment were randomly selected, cut into small pieces, chopped and mixed well with the help of a mixer. The resulting mixture, weighing 1.96 g, was homogenized in 10 mL extraction buffer (0.1 M potassium phosphate buffer, pH 7.0) and centrifuged under the same defined conditions to collect the supernatant for further investigation. Following that, three 200 mL beakers were prepared with 130 mM H_2O_2 substrate (pH 7.0) filled to a height of 50 mm and placed on heating plates set at 25 °C. The filter papers (Rundfilter MN 619 eh 18.5 cm, Macherey-Nagel GmbH & Co. KG, Düren, Germany) were cut into uniformly sized discs with a diameter of 2 cm. After soaking each filter paper disc in the mango liquid extract (supernatant), the disc was dried on a paper towel for five seconds to remove excess moisture. Each disc was placed at the bottom of the beaker with clean forceps, and the rising time of the disc was recorded in triplicate. The rate of enzyme activity (k) was calculated using the same defined equation 2. In addition, the rising time of the disc was converted to catalase units mL^{-1} using a calibration curve generated with known concentrations of catalase 1.20–24.00 units mL^{-1} (K-Catal, Romer Labs Deutschland GmbH, Butzbach, Germany), and the rising time of the disc was measured in six replicates (Figure 2). A power equation ($y = 569.21\ x^{-0.959}$, $R^2 = 0.988$) was fitted well, and the solution containing catalase $\leq$1.20 units mL^{-1} could not help the disc to float during the 3600 s of measuring.

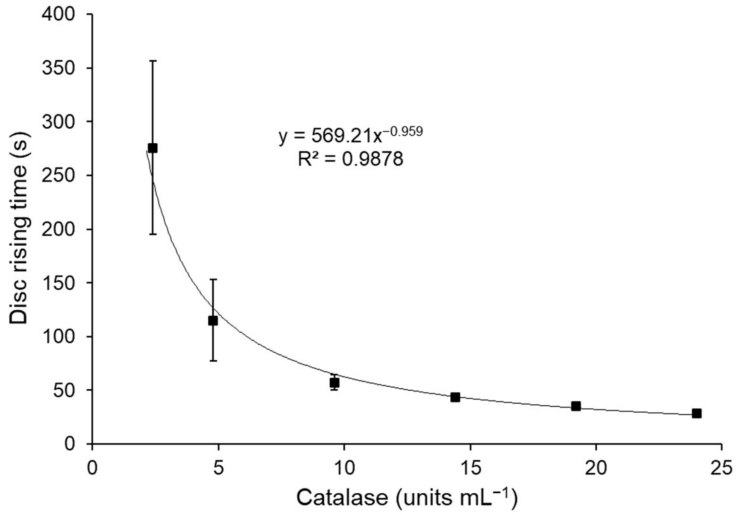

Figure 2. Calibration curve for disc rising time versus catalase units per mL.

2.4.2. Spectrophotometric Measurement

The spectrophotometric measurement of catalase activity was determined by following the method of Iwalokun et al. [30]. In this method, hydrogen peroxide (H_2O_2) was used as a substrate. The enzyme catalase can break down the H_2O_2 substrate into water and oxygen. The rate of decomposition of H_2O_2 is followed by observing the rate of decrease in the absorbance at 240 nm. The H_2O_2 substrate was prepared by mixing 50 g of 0.1M potassium phosphate buffer (pH 7.0) and 0.036 g of 50% (*w/w*) H_2O_2. The absorbance value should be

between 0.54–0.58 absorbance units. Six extractions were prepared for fresh and samples dried at 40 °C, 60 °C and 80 °C following the same procedure as described in indirect flotation test. The supernatant was collected and run separately in triplicate. For the activity assay, 100 µL of enzyme extract and 2.9 mL of H_2O_2 substrate were mixed, and the absorbance difference was recorded at 240 nm for 10 min at 25 °C using a spectrophotometer (DR-6000, Hach Lange GmbH, Berlin, Germany). The samples without enzyme extract were used as a control. The absorbance decrease rate of consumed H_2O_2 was converted to catalase units from a pure catalase standard curve prepared by running the defined units of catalase 0–24 units mL^{-1} (K-Catal, Romer Labs Deutschland GmbH, Butzbach, Germany) in triplicate against the same assay conditions (Figure 3).

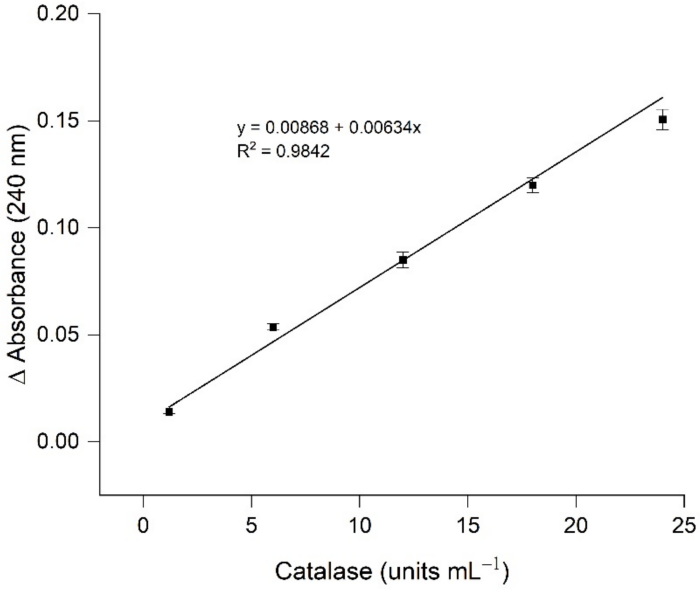

Figure 3. Calibration curve for absorbance versus catalase units per mL.

2.5. Statistical Analysis

The statistical analysis was performed by analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test using IBM statistical package SPSS 22.0. Statistically significant differences were indicated at $p < 0.05$. The data were plotted using Origin Pro 2020 (OriginLab Co., Northampton, MA, USA).

3. Results and Discussion

3.1. Color Changes

The CIELAB color parameters of fresh and dried mango slices are shown in Figure 4. The brightness (L^*) of the samples dried at a low temperature of 40 °C was well preserved with 67.77 ± 4.44 and close to the fresh samples with 68.39 ± 2.45. However, the L^* values decreased with increasing drying temperature and a significant ($p < 0.05$) difference in L^* values (62.02 ± 4.23) was observed when dried at 80 °C. This could be attributed to the fact that higher drying temperatures are associated with structural deformation of the fruit surface [1,15] and result in lower L^* values. In addition, it has been documented by many researchers that higher drying temperatures lead to the degradation of the heat-sensitive active compounds inside the fruit [10,23,31], which may be associated with a reduction in fruit brightness. A loss of brightness was also reported by Elamin O.M. [13] for conventionally oven-dried mango at 60 °C to 80 °C.

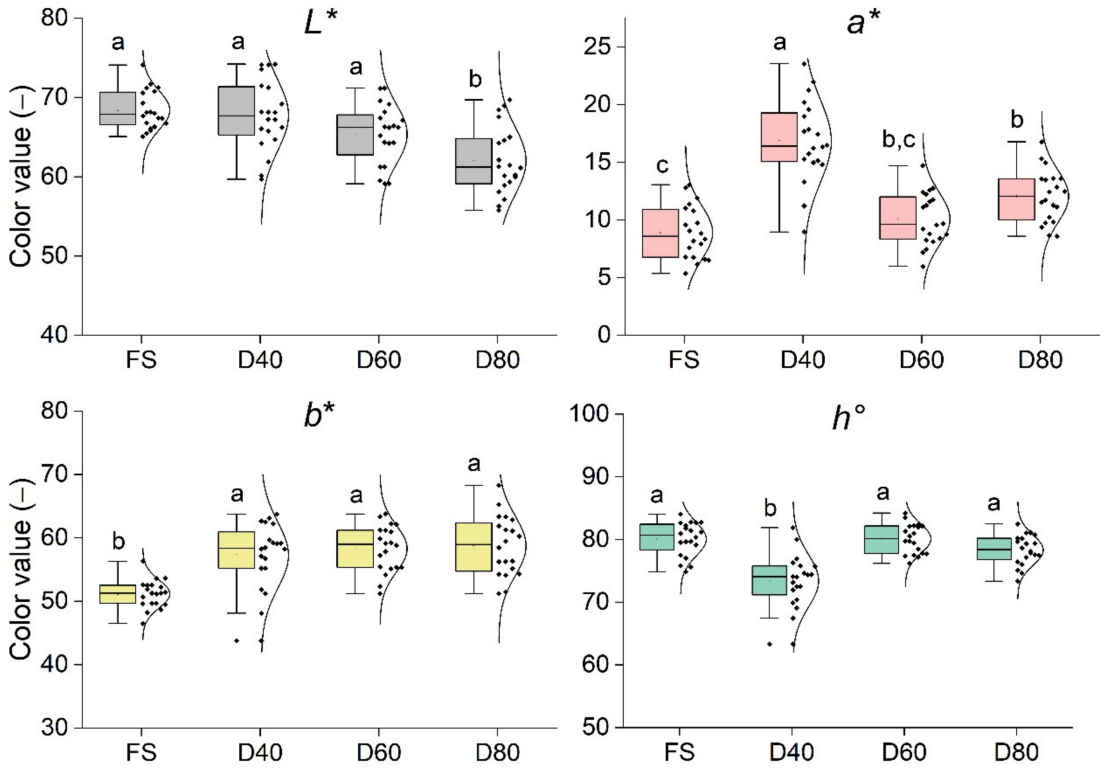

Figure 4. Color values: (*L**) lightness, (*a**) green-red, and (*b**) blue-yellow as well as (*h*°) hue angle of fresh and dried mango slices. FS = fresh samples and D = dried samples at temperatures of 40 °C, 60 °C and 80 °C. Different letters in a graph indicate significant differences at $p < 0.05$, ($n = 20$).

As for the redness parameter, the *a** value of 16.85 ± 3.53 increased significantly for mango slices dried at 40 °C compared to fresh mangoes with 8.92 ± 2.30. However, at an increased drying temperature of 60 °C, the *a** value decreased significantly to 10.09 ± 2.29 and approached the fresh samples; while at 80 °C, they increased again to 12.06 ± 2.26. The reason for this phenomenon might be related to the enzymatic and non-enzymatic browning of the fruits [32,33]. A higher *a** value at 40 °C may be due to the enzymatic browning reactions that normally occur when the enzyme polyphenol oxidase (PPO) comes into contact with the endogenous phenolic compounds during mango drying. Similar findings were also discussed in our previous study [7], where we observed the maximum PPO activity in mango slices dried at 40 °C. However, drying at 60 °C and 80 °C caused the PPO enzyme to deactivate or degrade, resulting in less enzymatic browning reaction. Following that, the higher *a** values at 80 °C seemed to be a result of brown polymers formed by the Maillard reaction [3,34].

The yellowness (*b**) of the dried mango slices increased significantly after drying, but no significant difference was observed between the different drying temperatures. The increase in *b** values due to drying could be related to the degradation of carotenoids and the formation of brown pigments by non-enzymatic (Maillard reaction) and enzymatic reaction [4,35]. The hue angle (h°) values are quite useful to express the visual impression of the samples. The lowest *h*°-values (73.43 ± 4.18) were obtained for mango slices dried at 40 °C compared to fresh samples (80.09 ± 2.69), and those dried at 60 °C (80.14 ± 2.34) and 80 °C (78.34 ± 2.40). This directly reflects the higher *a** value at 40 °C, as previously discussed, because there was no significant difference in *b** values at different drying temperatures.

In general, color is one of the most important sensory attributes of dried fruits that has a direct impact on quality perception and consumer acceptance [9,36]. However, considering color as a quality parameter to assess the quality of dried fruits is a rather fallacious decision, especially when preferences are mainly focused on the nutritional value of the dried fruits. For instance, as discussed in our results, no significant difference was

found in the hue angle of fresh mango slabs and those dried at 60 °C and 80 °C. However, when considering the nutritional value of dried mango under the given drying conditions, many studies have proven that drying at low temperatures could improve the preservation of the fruit's original components [1,7,16,20,37]. Furthermore, many pretreatment methods have been successfully developed to assist in minimizing colour change even during drying at high temperatures [11,12]. Therefore, preservation of color during drying is not a problem, while ensuring consumers that products are dried at a safe temperature with minimal nutrient loss is still an important issue. Considering all these aspects, the challenge is to develop a new quality prediction test that can distinguish between dried samples obtained at various temperatures.

3.2. Quality Prediction Test

The quality prediction test is directly based on the residual catalase activity in mango slices dried at 40 °C, 60 °C and 80 °C, which was assessed by flotation test and spectrophotometric measurements.

3.2.1. Flotation Test

Direct Flotation Test

In Figure 5A,B, mango slices dried at 40 °C show a shorter rising time 98.55 s and thus higher activity rate 0.69 mm s^{-1} compared to those dried at 60 °C (189 s, 0.44 mm s^{-1}) and 80 °C (1199.15 s, 0.05 mm s^{-1}), respectively. Therefore, it can be assumed that more catalase is retained in the dried mango slices when dried at a low temperature of 40 °C. An earlier study supports this assumption, where Mukhtar et al. [7] reported that the catalase degradation rate accelerated 3.84-fold during drying at 60 °C and 10.58-fold at 80 °C, compared to 40 °C.

Although this is a rapid and direct approach to differentiate between samples dried at 40 °C, 60 °C and 80 °C, there are certain limitations inherent in this method. This test is not suitable for fresh mango samples as they have a high moisture content, and the flesh of fresh mango can easily mix with the H_2O_2 solution. In addition, the pieces cut from the mango slices dried at 40 °C, 60 °C and 80 °C had different densities, as shown in Figure 5C. The density of the samples dried at 80 °C was higher (0.83 ± 0.02 g cm^{-3}) compared to those dried at 60 °C (0.76 ± 0.03 g cm^{-3}) and 40 °C (0.74 ± 0.06 g cm^{-3}), which may interfere with the flotation time. The increase in density at higher drying temperatures 80 °C could be due to excessive shrinkage [38]. Furthermore, it was noticed that air was trapped in some of the dried samples during hot-air convective drying, which could be due to the speed of air diffusion, water evaporation, change in pore size, rapture and dislocation of cells or splitting of the tissue [39]. Air entrapment in the dried samples could also influence the flotation time.

The surface alterations of dried mango, as illustrated in Figure 5D, could be another factor affecting the flotation time. The mango slices dried at 40 °C retained a more porous matrix with larger and randomly distributed pores, while those dried at 60 °C and 80 °C had a hard surface with mostly filled pores. Halder et al. [40] reported that during drying at temperatures below 50 °C, the cells remain intact, and water movement from the intracellular to intercellular space occurs only through microcapillaries and at a very slow rate. As a result, minimal deformation was observed. In contrast, when subjected to higher drying temperatures, the surface of samples was dried more quickly, causing it to become stiffer (case hardening) [15]. During the flotation test with direct-dried mango, the mango with a well-preserved porous structure may aid the rapid substrate diffusion and influence the floating time by reacting immediately with the available catalase.

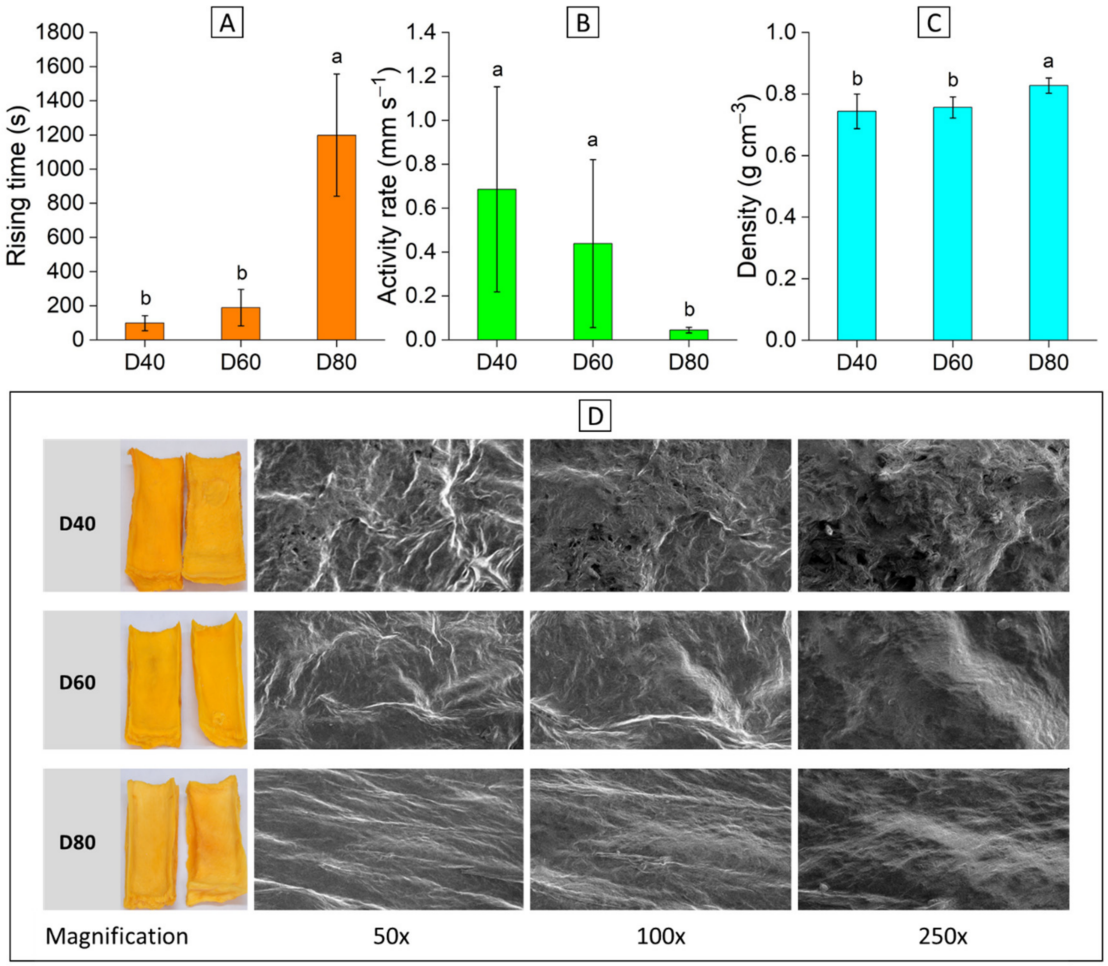

Figure 5. (**A**) Flotation time (s), (**B**) activity rate (mm s^{-1}), (**C**) density (g cm^{-3}) and (**D**) digital and SEM micrographs of the surface of mango slices dried at 40 °C, 60 °C and 80 °C. Different letters in a graph indicate significant differences at $p < 0.05$.

Indirect Flotation Test

The results of the indirect flotation test in Figure 6A,B show a significant difference between the disc rising time and the activity rate of mango slices dried at 40 °C (60.10 s, 0.89 mm s^{-1}) compared to 60 °C (543.13 s, 0.11 mm s^{-1}) and 80 °C (2403 s, 0.02 mm s^{-1}), respectively. This is particularly true for the extracted samples with more catalase units; they have a short disc rising time and a high activity rate [29]. Therefore, the catalase extracts obtained from mango slices dried at 40 °C exhibited a short disc rising time and high activity rate, closer to the fresh samples due to the higher retention of catalase activity 11.24 units mL^{-1}, as shown in Figure 6C. However, higher drying temperatures have a negative effect on the residual catalase activity [7] and consequently, the rising time of the disc increased, and the activity rate decreased. Moreover, some of the extracts from mango slices dried at 80 °C contained so few catalase units that they could not even support the disc to float within the measuring time of 3600 s, as listed in Appendix A Table A1. Hence, the indirect flotation test can be used as a screening test to distinguish dried samples treated at different temperatures. Furthermore, the indirect flotation test reveals a significant difference in disc rising time between mangoes dried at 40 °C, 60 °C and 80 °C, which the direct flotation test of dried mango failed to predict.

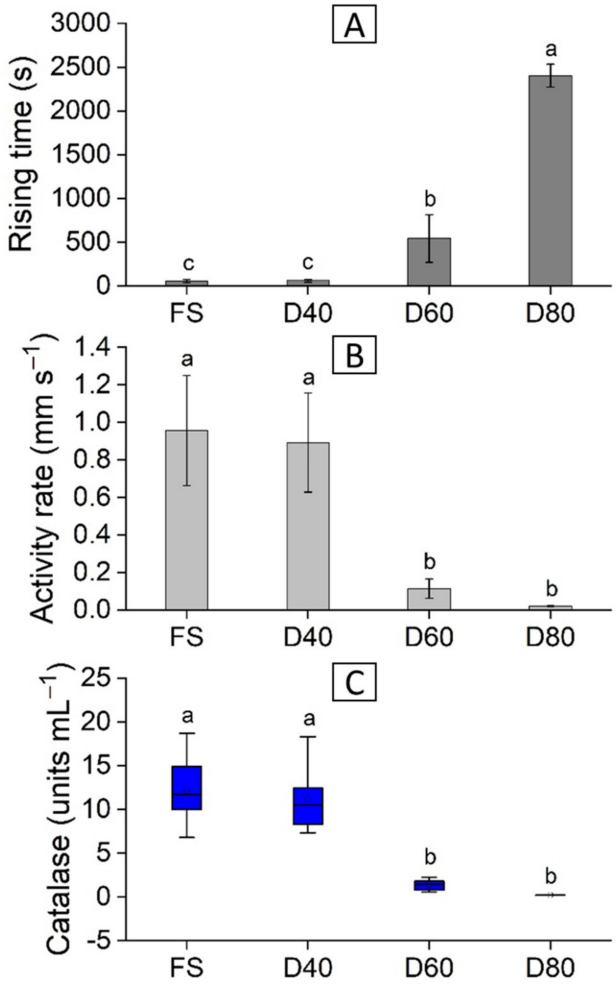

Figure 6. (**A**) Disc rising time (s), (**B**) activity rate (mm s^{-1}) and (**C**) catalase activity (units mL^{-1}) of fresh mango and mango dried at 40 °C, 60 °C and 80 °C. Different letters in a graph represent significant differences at $p < 0.05$, ($n = 10 \times 3$).

3.2.2. Spectrophotometric Measurement

In Figure 7, catalase activity in fresh mango extracts was found to range from 14.27–16.70 units mL^{-1}, with an absorbance difference of 0.096–0.111 arbitrary units (a.u.). However, the extracts obtained from mango slices dried at 40 °C exhibited a greater change in absorbance and higher catalase activity (0.113 a.u., 17.04 units mL^{-1}) compared to 60 °C (0.059 a.u., 8.20 units mL^{-1}) and 80 °C (0.021 a.u., 2.20 units mL^{-1}), respectively. According to Michaelis–Menten reaction kinetics, the reaction rate at a fixed substrate concentration, temperature and pH is directly related to enzyme concentrations. As the enzyme concentration increases, the reaction rate asymptotically approaches its maximum, and the absorbance difference also increases. Thus, the results confirm that the difference in absorbance values of mangoes dried at 40 °C, 60 °C and 80 °C is directly related to their residual catalase activity units. The mango with more catalase units decompose the H_2O_2 substrate at a faster rate, and as a result, a larger change in absorbance values was observed. Hence, by measuring the absorbance values, it's easy to differentiate between the dried samples treated at different temperatures. This approach is also simple, rapid and capable of detecting very low activity levels $\leq$1.20 units mL^{-1} that the flotation test was unable to detect. Furthermore, the results of catalase activity units for fresh samples and those dried at 40 °C are closely related for both spectrophotometric and indirect flotation tests. However, spectrophotometric measurements are more accurate for determining catalase activity in samples dried at 60 °C and 80 °C, as the indirect flotation test showed

a low accuracy for catalase activity below 5 units mL^{-1}. Therefore, spectrophotometric measurements are appropriate to deal with fresh samples and samples dried at different temperatures. Several researchers [41,42] have utilized the spectrophotometric measuring approach based on catalase activity in the field to investigate the biological samples.

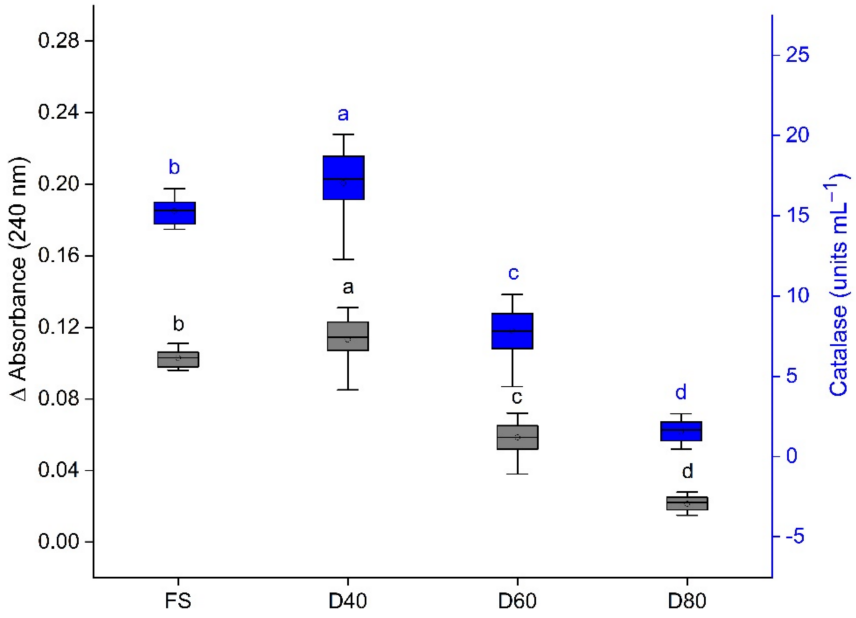

Figure 7. Absorbance and catalase activity (units mL^{-1}) of fresh mango and mango dried at 40 °C, 60 °C and 80 °C against the fixed concentration of 0.036% H_2O_2 substrate (initial absorbance 0.54–0.58 a.u.) for 10 min at 25 °C. Different letters indicate significant differences for absorbance values and catalase activity separately at $p < 0.05$, ($n = 6 \times 3$).

4. Conclusions

Catalase activity in dried mango slices can be used as a preliminary screening test to predict quality degradation caused by excessive heat exposure during drying. Results proved that the catalase activity test could easily distinguish between dried mango slices treated at low or high temperatures, which is hard to detect based on color. To assess catalase activity in dried mango slices, we achieved promising results using the spectrophotometry and indirect (disc) flotation test compared to the direct flotation test of dried mango. Here the spectrophotometric analysis offers even more sensitive measurements than the indirect flotation test. Both spectrophotometry and indirect flotation method are simple, rapid, cost-effective, require little equipment and can be easily adopted in food testing labs or the fruit drying industry. Further studies should focus on the correlation of the catalase enzyme with heat-sensitive nutrients in mango. It is also important to understand the effects of storage time and packaging materials on the stability of the enzyme. Moreover, the catalase enzyme is present in almost all fruits, vegetables and nuts, so this study could be extended to evaluate the quality of other dried products in the future.

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Appendix A

Table A1. Disc rising time (s) and activity rate (mm s^{-1}) for fresh mango and mango dried at 40 °C, 60 °C and 80 °C in 130 mM H_2O_2 solution (pH 7.0) at a height of 50 mm at 25 °C.

No. of Catalase Extracts	Time for the Disc to Rise (s)				Activity Rate (mm s^{-1})			
	FS	D40	D60	D80	FS	D40	D60	D80
1	48.33	66.67	259.33	-	1.03	0.75	0.19	-
2	62.33	50.67	322.33	-	0.80	0.99	0.16	-
3	38.67	67.00	325.33	-	1.29	0.75	0.15	-
4	59.33	74.67	954.67	2449.67	0.84	0.67	0.05	0.020
5	62.67	53.67	512.67	-	0.80	0.93	0.10	-
6	42.67	53.00	318.00	2266.67	1.17	0.94	0.16	0.022
7	90.67	84.00	947.67	2333.33	0.55	0.60	0.05	0.021
8	83.67	75.00	721.67	-	0.60	0.67	0.07	-
9	49.00	41.33	723.00	2562.33	1.02	1.21	0.07	0.020
10	34.33	35.00	346.67	-	1.46	1.43	0.14	-

FS = fresh samples, D = dried samples. (-) the disc did not rise within the waiting time of 3600 s.

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