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Original Article

Biochemical Dynamics and Quality Attributes of Strawberry Fruits across Maturity Stages with Respect to Different Preservation Methods

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ABSTRACT

Strawberry is nutritious but perishable, and it is susceptible to quality flaws and infections after harvest. **Objective:** To examine strawberry biochemical changes with harvest maturity and preservation methods. **Methods:** The experiment was factorial with a completely randomized design (CRD). Strawberry cv. Chandler fruit was harvested at three color-based maturation phases (M1: 0% red, M2: 50% red, and M3: 100% red), frozen, and freeze-dried and analyzed for quality. Total soluble solids (TSS), titratable acidity (TA), vitamin C, total phenolic content (TPC), total antioxidant capacity, SOD, POD, CAT, and organoleptic characteristics were assessed for quality. **Results:** Due to harvest maturity, completely ripe strawberries displayed higher biochemical properties such as total soluble solids (TSS), titratable acidity (TA), and TSS/TA than 50% and 0% of red strawberries. 100% red strawberries also had greater TPC and POD. Compared to strawberries picked during the green stage (0% red color), strawberries harvested at 100% and 50% red color had better aroma, color, and flavor. TSS, TSS/TA, Vitamin C, antioxidants, total phenolic content, and peroxidase were better in freeze-dried strawberries than frozen strawberries. **Conclusions:** The superior fruit aroma and color rating made frozen strawberries more popular than freeze-dried ones. The preserved fruit retained these qualities better for 7 months in dried storage. This study found that strawberry fruit should be harvested at 100% red and freeze-dried for commercial use.

INTRODUCTION

Strawberry (*Fragaria ananassa*) is a popular and economically significant fruit all over the world. This is a widely consumed fruit known for its rich nutrient profile and vibrant flavor [1-2]. Vitamins, minerals, and phytochemicals make them popular in culinary and health aspects [3]. Due to their vitamin C, folate, and phenolic content, strawberries are rich in bioactive compounds. Strawberries deteriorate soon after harvest. Strawberry postharvest life is brief due to its delicate structure and intense respiration; it bruises readily and has fungal infections [4-5]. This affects bioactive components and antioxidant activity. Thus, proper preservation is essential for quality and shelf life. Their fragility and short shelf-life hinder storage and preservation. Shelf life was extended in several ways. Three strawberry types were studied for postharvest flavor and durability [6]. After harvest, CO_2 treatments modify fruit color for 9 days. When exposed to 20 kPa CO_2 at a flow rate of 150 ml/min, the shelf life of strawberries was prolonged by 4 days. Packaging materials maintain the postharvest quality and storage life of horticultural commodities [7]. It was found that all fruit samples survived well after storage, except for

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strawberries in modified atmosphere packaging (MAP), which tasted softer after 9 and 12 days [8]. To extend strawberry fruit shelf life, controlled-environment storage was widely used. Gas composition affected total soluble solids (TSS), titratable acidity (TA), aroma, and consumer preference [9]. Using 10% CO2 and 11% oxygen extended strawberry shelf life and maintained quality metrics. For strawberry fruit shelf life, calcium nitrate (0.5, 1.0, and 2.0%), calcium chloride (0.05, 0.10, and 0.20%), and ascorbic acid (0.01, 0.02, and 0.05%) were tested [10]. Calcium chloride (0.05%) improved the shelf life to 9 days. This effect was influenced by lower acid, moderate TSS, and more sugar. Frozen fruit lasts longer and tastes fresh. Due to enzyme activity below freezing, frozen fruits and vegetables lose color and flavor [11–12]. Fruit drying is another simple and reliable preservation method that has been used for centuries. Freeze-drying stiffens the product, preventing solute and liquid mobility. To maximize fruit post-harvest life and quality, one must understand biochemical changes throughout maturation and how they combine with preservation strategies. A sustainable postharvest preservation solution is needed to extend the shelf life of strawberry fruit.

This study assessed strawberry quality using various preservation methods. We researched how sugars, acids, and antioxidants affect strawberry quality during growth. The study also investigated strawberry preservation procedures to see which ones best preserve or increase nutritional and sensory qualities at different maturation stages. This research will examine strawberry preservation methods to boost the quality and shelf life of strawberry fruit.

METHODS

Fruit Material and Experiment Layout

Fresh, uniform-sized, and healthy strawberry field samples were obtained from the Institute of Horticultural Sciences (IHS), University of Agriculture, Faisalabad (UAF) at three harvest maturation stages (M1, M2, and M3) based on red color development. At IHS, UAF, a completely randomized design (CRD) with two factorial configurations and three replications (10 berries per replicate) was used. Harvest maturity (M1: 0%, M2: 50%, and M3: 100%) and preservation method (P1: frozen and P2: freeze-dried) were examined. Fruit samples were preserved using P1 and P2 according to the experimental configuration. The initial preservation technique involved freezing strawberries in LDPE zip bags that were tagged with harvest ripeness and three replications and stored at a temperature of -20°C. For freeze-drying, strawberries were washed, half-sliced, and placed in the freezer drying chamber for 7 months for further analysis.

Data Collection

The data were collected for various biochemical attributes (total soluble solids, titratable acidity, ascorbic acid, total phenolics, antioxidants, and enzymatic contents) and the organoleptic characteristics (aroma, color, and flavor) at 0 days and freezing and freeze drying after 7 months of storage.

Total Soluble Solids (TSS)

To examine TSS, the strawberry juice from each replication of three maturity stages fruits was used, and two readings were noted from each replication by using the Digital Refractometer Model No. ATAGO, RX 5000, Japan. The mean value of each replication was calculated in °Brix.

Titratable Acidity (TA)

To evaluate TA 10 mL strawberry juice was added to a beaker with 20 mL distilled water. After mixing, 2–3 drops of phenolphthalein were added and shaken. The resulting solution was dropwise titrated against 0.1 N NaOH till pink. The following formula was used to measure TA in percentage [13].

$$TA(\%) = \frac{A \times 0.0067}{B}$$

Where;

A = Volume of NaOH used (mL)

B = Strawberry fruit juice (mL)

TSS: TA

Dividing the amount of TSS by the value of TA allowed us to determine the ratio of TSS to TA for each treatment.

Ascorbic Acid

The amount of ascorbic acid in strawberry fruit was calculated by adding 10 ml of juice extracted from each sample to 90 ml of a 0.4% solution of oxalic acid in a measuring flask. The solution was filtered using Whatman® No. 1 filter paper. Then, a 5 mL aliquot of each sample was titrated against 2,6-dichlorophenol indophenol dye until the color changed to pink and was expressed in units as mg100 g^{-1}FW [14].

Total Phenolic Content (TPC) and Total Antioxidant Content (TAO)

Strawberry fruit total phenolic content (TPC) was measured in mg 100 g^{-1} FW [15]. Using a pestle and mortar, 1g frozen strawberry fruit pulp was homogenized with 5 mL extraction liquid. The extraction combination comprised CH_3OH, acetone $(CH_3)_2CO$, and HCl (10:8:2). SHIMADZU, UV-1800 240V centrifuged the solution for 5 min at 4°C after homogenizing it at 14000 rpm. The pellets were discarded and the supernatant analyzed. The supernatant (100 μL) was carefully mixed and vortexed with 200 μL FC-reagent (Folin-Ciocalteu After adding 800 μL of 700 mM sodium carbonate (Na_2CO_3), the liquid was vortexed again. The spectrophotometer measured reaction mixture absorbance at 765 nm after 1h. Strawberry fruit's TAO

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activity was quantified using DPPH scavenging [16]. 1 gram of frozen, preserved strawberry pulp was ground in a 5 mL extraction mixture with a pestle and mortar. The extraction solution was 10:8:2 methanol, acetone, and HCl. After homogenization, the mixture was vortexed and centrifuged at 4°C for 5 minutes using a centrifuge. The solid particles were discarded, and the liquid was used to test antioxidants. A 0.004% NPPH solution in 5 mL was mixed with 50 µL of supernatant and sealed with aluminum foil. After 30 minutes, the spectrophotometer was set to 517 nm to measure sample absorption. Antioxidant activity was measured using DPPH scavenging.

Enzymatic Analysis

The samples were subjected to enzyme extraction using the prescribed methodology [16]. This was achieved by homogenizing 1g of strawberry flesh in a 2-milliliter phosphate buffer solution and centrifuging at 10,000 g for 10 minutes. The supernatant was used to assess Superoxide Dismutase (SOD), Peroxidase (POD) and catalase (CAT) activities. The 560 nm nitro blue tetrazolium method was used to detect SOD activity. The change in absorbance at 470 nm during tetraguiacol production from guaiacol was used to evaluate POD activity. CAT activity was identified by measuring hydrogen peroxide degradation at 240 nm. Enzyme activity was measured in U mg^{-1} protein units.

Organoleptic Characteristics

The organoleptic qualities are comprised of aroma, color, and flavor. Based on sensory assessment, these qualities were evaluated. A panel of 10 judges was asked to assess the organic acceptability of strawberry fruit using the following modified hedonic scale for this purpose:

1. I dislike it very much.
2. Dislike moderately
3. Neither like nor dislike
4. Like moderately
5. Like extremely

Statistical Analysis

Statistix 8.1 software was used to analyze the data under CRD for two factors: harvest ripeness and preservation procedures. Treatment significance was assessed using an ANOVA table. LSD tests at 5% significance were performed on all pairwise combinations [17].

RESULTS

TSS, TA, and TSS/TA

TSS in strawberry fruit varied significantly with maturity (Fig. 1A). Strawberry with 100% red color (12.66 °Brix) has a far higher Brix rating than strawberry with 0% red color (7.75). However, the TSS of frozen and freeze-dried strawberry fruit varied greatly after 7 months. The freeze-dried strawberry had the greatest TSS (11.4 °Brix) and the frozen strawberry the lowest (10.93). A 100% red strawberry freeze-dried product has the highest TSS. At different maturity phases, strawberry TA varied significantly (Fig. 1B). The strawberry with the greatest value (0.76%) was 0% red, while the strawberry with the lowest value (0.46%) was 100% red. There was no difference in strawberry TA when frozen or freeze-dried. Under two preservations, freeze-dried strawberries had the highest value (0.73%) and frozen strawberries the lowest (0.42%). Strawberry fruit's TSS/TA ratio varied considerably during development (Fig. 1C). Strawberry fruit with 100% red color had the greatest TSS/TA ratio (27.45), and fruit with 0% red color had the lowest (10.16). Strawberry fruit kept by freezing or freeze-drying had varied TSS/TA ratios. Freeze-dried strawberries had the greatest TSS/TA value (25.83) at maturity 3, whereas frozen strawberries had the lowest (25.68).

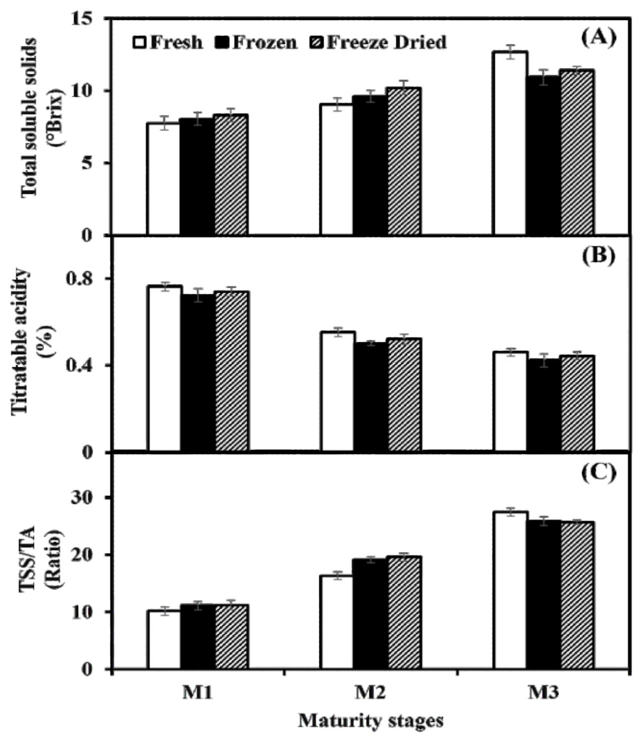

Figure 1. Effect of Maturity Stages and Preservation Methods on TSS (A), TA (B), and TSS/TA (C) of Strawberries.

Ascorbic Acid, TPC, and TAO

Strawberry ascorbic acid content did not much vary with maturity (Fig. 2A). At various maturation phases, strawberries with 50% red color had the highest ascorbic acid concentration (91.27 mg 100 g^{-1} FW), while 100% red and 0% red had the lowest (89.43 and 90.44 mg 100 g^{-1} FW). At maturity 2, the freeze-dried strawberry had the most ascorbic acid (78.63 mg 100 kg^{-1} FW) and the frozen strawberry the least (75.89 mg 100 kg^{-1} FW). The TPC of strawberry fruit varied dramatically with maturity (Fig. 2B). The strawberry fruit with the lowest TPC (2586.9mg 100 g^{-1} FW) was 50% red, whereas the strawberry with the highest

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TPC (2880.80) was 0% red. Freeze-dried strawberries had the highest TPC (3162.9 mg 100 g^{-1}FW) at the 3rd maturity stage, whereas frozen strawberries had the lowest (2499.6mg 100 g^{-1}FW) at the 2nd maturity stage. After freeze-drying, the 100% red strawberry had the highest TPC, whereas the 50% red strawberry had the lowest. Strawberry antioxidant capacity changed slightly with maturity (Fig. 2C). The strawberries with the greatest grade (91.62) and the lowest (88.85) were 0% and 100% red. The first maturity stage of frozen strawberries had the highest value of 91.95, while the third maturity stage had the lowest. Antioxidant capability and fruit ripeness and preservation were found to interact significantly.

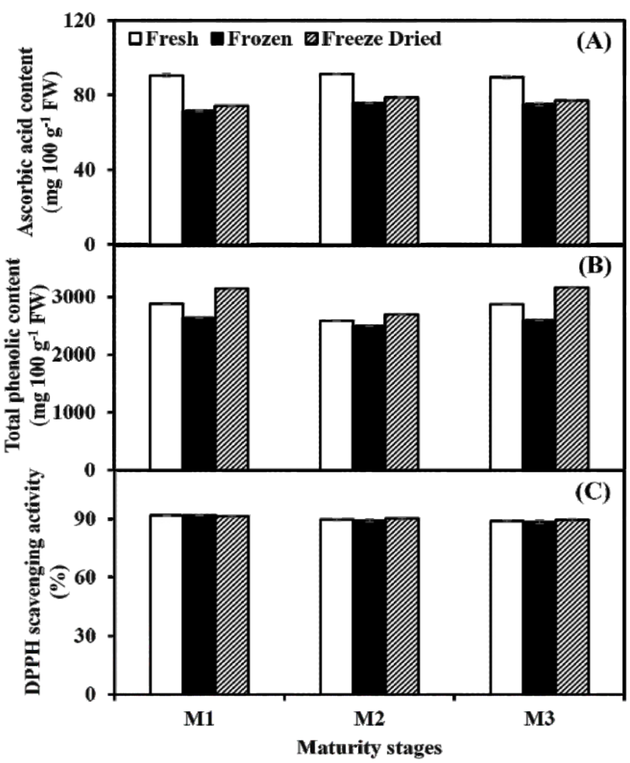

Figure 2. Effect of Maturity Stages and Preservation Methods on Ascorbic Acid Content (A), Total Phenolic Content (B), and DPPH Scavenging Activity (C) Of Strawberries.

Antioxidant enzyme activity

At different developmental stages, strawberry fruit SOD activity interacts significantly (Figure 3A). Strawberry fruit SOD relationship with ripeness and preservation was significant. The freeze-dried strawberry with 0% red content had the highest protein concentration (8.63 U mg−1Protein), whereas the strawberry with 50% red content had the lowest (6.37 U mg−1 Protein).

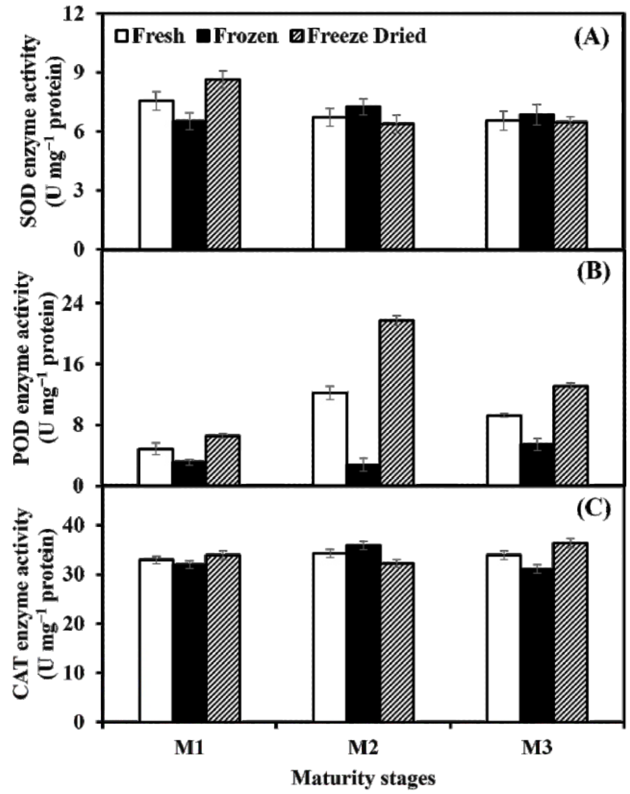

Figure 3. Effect of Maturity Stages and Preservation Methods on SOD(A), POD(B), And CAT(C) of Strawberries.

Strawberry fruit peroxidase varied significantly during development (Figure 3B). The strawberry with 0% red color had the lowest POD activity (4.85 U mg^{-1} protein), while the strawberry with 50% red color had the highest (12.2 U mg^{-1} protein). At maturity level 2, freeze-dried strawberries exhibited the highest POD activity (21.74 U mg^{-1} protein), while frozen strawberries had the lowest (2.76 U mg^{-1} protein). Strawberry fruit catalase activity did not alter with maturity (Figure 3C). The CAT activity of frozen and freeze-dried strawberry fruit did not differ statistically. The strawberry fruit ripeness and preservation-CAT activity relationship was non-significant. The lowest CAT activity was found in 0% red strawberries and the greatest in 50%. Freeze-drying preserved 100% red strawberry CAT activity better than freezing strawberries.

Organoleptic Characteristics

Aroma is a popular fruit quality. Sensory testing reveals strawberry and other fruit aromas. Strawberry aroma varied greatly with ripeness (Figure 4A). In terms of aroma, 100% red strawberries scored 7.2, while 0% red strawberries scored 2.87. Frozen strawberries scored 7.25, whereas freeze-dried strawberries scored 5.14. Strawberry fruit development, preservation, and aroma interaction were non-significant. Strawberry fruit color varied greatly during development (Figure 4B). The 100% red strawberry scored 7.84, while the 0% red strawberry

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scored 3.91. Strawberry fruit preserved by freezing and freeze-drying had various hues. Strawberry freeze-dried scored the lowest (5.22) and frozen the highest (8.55). Strawberry flavor changed greatly with maturity (Figure 4C). The maximum color grade was 8.07 for 100% red strawberries, while the lowest was 3.72 for 0% red strawberries. Strawberry fruit preserved by freezing and freeze-drying tasted similar. No significant effects of fruit maturity and preservation on flavor were found.

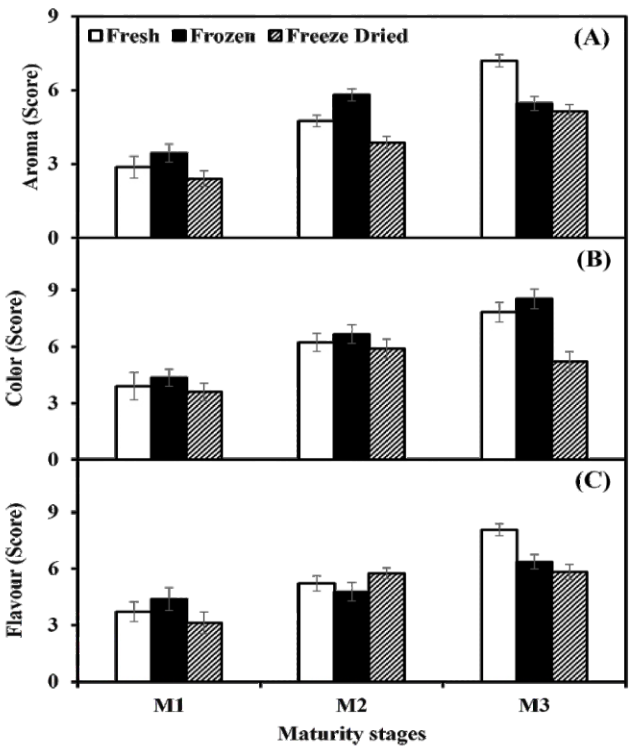

Figure 4. Effects of maturity stages and preservation methods on the aroma (A), color (B), and flavor (C) of strawberries.

DISCUSSION

Fruits smell different from fresh and stored fruits depending on acidity. Citric and malic acids dominate fruit acidity [18-20]. Same is the case with our stored fruit, strawberry lose acid over storage time about (0.43%), but decrease below a critical threshold can lead to poor quality. Complex maturation and ripening processes alter sugars and organic acids [21]. TSS increases with fruit ripening. Freeze-dried strawberries have the highest TSS at M3(11.4 °Brix) as compared to other maturity stages. Moisture also increases TSS. Low-soluble solids and high acid levels early in fruit formation make strawberries sour [22–24]. In our results strawberries at M1 have sour taste because of this phenomenon. Sugar increases as fruits ripen and mature because their metabolisms require various acids [25]. Strawberries contain ascorbic acid, anthocyanins, and flavanols [26]. When strawberries are plucked, it impacts their ascorbic acid value. Ascorbic acid degrades with age due to instability as in our frozen strawberries after harvesting (75.89 mg 100 kg^{-1} FW) [27]. Strawberry phenolics vary by cultivar, growing conditions, maturity, and post-harvest care [28]. Vitamin C and phenolic compounds are examples of fruit antioxidants that possess the ability to scavenge oxygen radicals. [29]. Strawberry antioxidant activity in our study was (88.85), whichexceeds apples, peaches, grapes, and others [30]. In a study of over 1,000 foods and beverages, strawberries rated third in TAO per serving [31]. Scientists found that peaches lose SOD after storage, which supports our findings as the same trend in strawberries up to (6.37 U mg^{-1} Protein) [32]. Red strawberries lose peroxidase activity quickly, but white ones boost it [33]. Peroxidase and polyphenol oxidase activity drop by 80% during fruit ripening [34]. In our study the frozen strawberry POD activity was lowest (2.76 U mg^{-1} protein). Biochemical strawberry fruit changes produce ROS like O2, H2O2, and OH, causing oxidative stress [35-36]. Strawberry harvest dates depend on surface color, attractiveness, firmness, and nutritional content. Strawberry size and color are essential visual attributes [37]. Anthocyanin concentration was low during growth, but it increased significantly in three days as it ripened. Anthocyanins color fruit, but sugars, acids, and polyphenols flavor it [38]. Variety, or genotype, evolves qualitatively and quantitatively at maturity. Sugars and acids alter fruit nutrition and taste [39]. Our findings support these results as strawberries with 100% red color have higher rating of aroma (7.2), color (6) and flavor (7) rating. This study used rigorous testing and analysis to provide a comprehensive understanding of the intricate biochemical mechanisms taking place in strawberries as they undergo various phases of ripening. Additionally, it investigated the impact of preservation techniques on these processes. This study held great importance in terms of enhancing our comprehension of fruit physiology and offering valuable insights into the optimization of preservation techniques to maintain its qualitative characteristics, including flavor, color, and nutritional composition.

CONCLUSIONS

This study emphasizes harvesting strawberries at full ripeness (100% red color) for superior biochemical characteristics. Freeze-drying is the preferred preservation method, preserving essential attributes better than freezing. Although consumer preference leans towards frozen strawberries, the study recommends freeze-drying for optimal commercial harvest and preservation, ensuring the longevity of desirable attributes for up to 7 months of freeze-dried storage. This research provides valuable insights and practical recommendations

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for maximizing nutritional quality and shelf life in the strawberry industry.

Authors Contribution

Conceptualization: AAK
Methodology: MMA
Formal analysis: AAK, RL, ZU
Writing-review and editing: ZU, WA, AM, AAT, MMA

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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