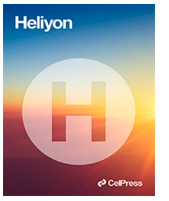
Heliyon 9 (2023) e18358

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Analytical and bioluminescence-based non-invasive quality assessment of differentially grown strawberry (*Fragaria x ananassa* Duch. 'Asia') during household refrigeration storage

Ildikó Jócsák [a,*], György Végvári [b], Kristóf Klász [a], Gabriella Andrássy-Baka [c], Katalin Somfalvi-Tóth [a], Éva Varga-Visi [d]

[a] *Department of Agronomy, Institute of Agronomy, Hungarian University of Agriculture and Life Sciences, Kaposvár Campus, Guba Sándor Street 40, H-7400, Kaposvár, Hungary*
[b] *Food and Wine Research Institute, Eszterházy Károly University, 6 Leányka Street, H-3300, Eger, Hungary*
[c] *Hungarian University of Agriculture and Life Sciences, Kaposvár Campus, Guba Sándor Street 40, H-7400, Kaposvár, Hungary*
[d] *Department of Physiology and Animal Health Institute of Physiology and Nutrition, Hungarian University of Agriculture and Life Sciences, Kaposvár Campus, Guba Sándor Street 40, H-7400, Kaposvár, Hungary*

ARTICLE INFO

Keywords:
Strawberry
Refrigeration storage
Antioxidant capacity
Lipid oxidation
Ultra-weak bioluminescence

ABSTRACT

Strawberry is a typical spring fruit, for which consumer demand is particularly high; however, information is scarce on the comparison of quality traits of strawberries of local and imported origin during household refrigeration storage. That is why we sought to answer the question of to what extent the quality of strawberries changes during 5 days of household refrigeration storage. The choice of methods was focused on fast and at the same time informative analytical methods: pH, total antioxidant capacity, and lipid oxidation. Furthermore, a non-invasive imaging technique: ultra-weak bioluminescence measurement was carried out along with the determination of fruit morphological (weight, height, width, and color) and analytical (antioxidant capacity and lipid oxidation) traits. The data indicate that the longer strawberries are cultivated in the same plantation, the smaller their fruit weight and the lower their fruit quality become.

In addition, the results cast light on a controversial fact: despite the fact that the sample from the store had the finest appearance, as determined, its antioxidant capacity was the lowest for the duration of home refrigeration storage, indicating the lowest nutritional value.This was validated by the lipid oxidation levels, which were defined by the amount of malondialdehyde as well as by the rate of ultra-weak bioluminescence. The research underlines the exceptional value of local fruits over importand provides valuable information to customers, encouraging them to purchase strawberries from local farms in order to not only support the local economy but also to adopt a more health-conscious attitude. In addition, ultra-weak bioluminescence testing offers a non-invasive method for assessing fruit quality.

1. Introduction

The global consumption of berries indicates an upward tendency. Strawberry (*Fragaria x ananassa* Duch.) is a popular spring fruit

* Corresponding author.
E-mail address: jocsak.ildiko@uni-mate.hu (I. Jócsák).

https://doi.org/10.1016/j.heliyon.2023.e18358
Received 24 March 2023; Received in revised form 13 July 2023; Accepted 14 July 2023
Available online 15 July 2023

that is in high demand among consumers [1], since it is the first fresh fruit that local farmers can provide to consumers following the winter months. Strawberry fruits contain health-promoting compounds, such as antioxidant phenolic compounds, vitamins, and numerous minerals, including potassium, magnesium, phosphorus, and iron. Due to its beneficial nutritional properties, fresh consumption of strawberries stimulates stomach and intestinal function [2], but as a non-climateric fruit, the condition of the fruit at harvest is of paramount importance because the quality of the fruit undergoes constant decay after harvest [3].

The sensory qualities of fruits affect customers' ratings of freshness [4], so fresh, undamaged strawberries with the most nutraceuticals are preferred. This gave rise to the special interest in the differences between imported and local fresh fruits [5] that has been encountered in the study of fresh market fruits' qualitative properties as well. In a German case study, Bhat et al. [5] examined preferences of strawberry consumers and concluded that customers preferred blemish-free strawberries, yet 43% still accepted strawberries with apparent squashy patches, showing that appearance is not the main driver of consumer preference. Among UK consumers [6], local foods are preferred over imported ones. According to the reviewed literature, many analytical and instrumental strawberry quality evaluation studies seek to address problems before and after packaging, transportation, and shelf storage and to increase the shelf life of strawberries in supermarkets [7]. However, there is a lack of information about how the nutritional and sensory qualities of strawberries change during home storage in the refrigerator, where sophisticated storage instrumentation, circumstances, and methodologies cannot be used but which changes unavoidably affect strawberry nutritional and sensory values. Chisari et al. [8] examined the cultivars 'Madame Moutot' and 'Elsanta' after 4 °C storage and found a correlation between polyphenol oxidase and peroxidase enzyme activity and browning rate as a quality indicator. Péneau et al. [4] examined how firmness, soluble solid content, and titratable acidity affect consumer and expert freshness evaluations. Pavlovska et al. [9] examined how temperature and storage period affect strawberry vitamin C concentration, including 4 °C refrigeration and foungd that the samples lost vitamin C over 11 days of refrigerated storage.

Non-destructive food quality determination is of great importance in food technology research, among which ultra-weak bioluminescence (UWLE) has been used as a detection tool for the identification of abiotic [10] and biotic [11] stressors in plants, as well as for food quality and safety assessment [12–18]. In their review work, Nematollahi et al. [12] found UWLE measurements to be effectively used for the determination of tomato maturity stages, quality of sweet potato, and also there was correlation found UWLE signal intensity and the viability and germination rate of rice and coffe seeds. The basis of these investigations is that reactive oxygen species (ROS), formed during food aging react with biomolecules, such as lipids, proteins, and even DNA, resulting in the formation of unstable intermediers, during which ultra-low intensity photon emission is generated [13] in the form of bioluminescence. This phenomenon, which can be monitored by a sensitive charge coupled device (CCD) camera and visualized via proper software supplementation [14], provides information about the organism's oxidative state, which is, in the case of fruits, related to the maturity state and/or quality traits [12,15]. Altemimi et al. [16] investigated food contact surface microbial contamination, tested the efficiency of sanitizers via ATP-bioluminescence method and successfully detected *Escherichia coli* and *Staphylococcus aureus* on surfaces and tools also *Pseudomonas aeruginosa* [17]. Also, ATP-bioluminescence sensor and plate counting techniques produced comparable results thus make this techniquie possible for on-site measurement as well [18].

Since no studies have specifically compared the quality characteristics of locally grown and imported strawberry samples during home refrigeration storage, we decided to monitor the quality characteristics of fresh and stored samples of various cultivation and origin. The fresh samples came from a local farm that used strawberries of varying maturities and cultivation methods, while the preserved samples came from a grocery store. Neither study has compared the qualitative characteristics of locally grown versus imported strawberries during home refrigeration storage.

The selection of methods was centered on two key areas: non-destructive methods, such as UWLE measurement, as well as fruit weight, height, width, and color; and rapid analytical methods, such as pH, total antioxidant capacity, and lipid oxidation parameters [19–21]. To our knowledge, no previous study has investigated UWLE on strawberries during home refrigeration storage; therefore, we sought to determine the effect of 5 days of cool storage at 4 °C on strawberry biophoton emission. The results indicate that elder strawberry plantations underperform their younger counterparts in terms of both size and quality. All alterations were detectable via conventional methods and a novel, ultra-weak bioluminescence-based assay, which may contribute to the development of rapid fruit quality testing methodologies, as well as raise health preservation awareness regarding the consumption of local fruit products.

2. Materials and methods

2.1. Plant material

In our work we used a commercially available variety of strawberry: 'Asia'.

> 'Asia'. The berries are large (weighing 60–80 g, conical shape, 28–35 g) and bright red. Its fruit is conical, large and has a very intensive aroma. The flesh is pale red, sweet, juicy, moderately dense. Medium early variety, easy to pick and very productive. The color of the fruit is dark red, suitable for intensive growing conditions and home gardens. Also suitable for fresh consumption and processing. Planting time: August–September. It prefers a sunny semi-shady place, the soil should be rich in nutrients and must have good water permeability.

2.2. Experiment location, plant growth, nutrient supply and plant protection

The farm is located in Lengyeltóti, Somogy county, Hungary. After proper soil preparation and covering with black ground cover

foil and the area was irrigated through drip tape. A++ plantlets were planted in single rows, 16 cm apart. Time of plantings: 'Asia' 2018: 09–08–2018; 'Asia' 2019: 08–08–2019; 'Asia' 2020 open field: 19–03–2020; 'Asia' 2020 greenhouse (GH): 19–03–2020.

Nutrient supply: through dripping tape and foliar fertilization. As a basic principle, in addition to an even nitrogen supply, phosphorus-predominant fertilizer was provided in the spring period, and potassium-predominant fertilizer at the time of ripening with high active ingredient content, perfect and fast dissolution, completely free of sodium, chlorine and carbonates, low EC, and EDTA-chelated microelements.

The frequency of irrigation was determined by the soil moisture content and averaged as 2 L/m^2. Irrigation was performed weekly in February–March and during the period of intensive growth in spring, then in every two days during harvest.

Plant protection was implemented following the principle of reduced chemical use. Among the agrotechnical methods, the annual plant protection started with the mechanical foliar removal in the spring and after the harvest, as the infected leaves were removed during this process.

Immediately after flowering, protection against strawberry botrytis fruit rot (*Botrytis cinerea* Pers.) was realized with fungicide 'Quadris'. No chemical plant protection treatment was applied from the end of flowering to the end of the harvest period. Among the pests, mites were protected with the sulfur-containing agent 'Thiovit Jet' and aphids with the absorbent agent 'Karate Zeon 5 CS' was used. Leaf fertilization was carried out at the same time as with 'Amalgerol®' along with the plant protection treatment.

2.3. Meteorological data description and acqusition

Daily meteorological data was provided from the homogenized and gridded dataset of the Hungarian Meteorological Service. The distance between the nearest grid point and the discussed location in Öreglak is 6 km, what is appropriate to study the local weather properties due to the simple topography of the area.

The grid point dataset was derived from measured data on ISO-standard meteorological stations in accordance with WMO requirements all over Hungary. First, the data series went through a serious quality control procedure, then the dataset was homogenized and completed by the MASH homogenization method [22]. Lastly, the controlled, homogenized and complete data series were interpolated by the MISH interpolation method [23] to a 0.1°x0.1°grid. The temporal coverage of the dataset is 1971–2020 containing daily meteorological data. Available parameters are daily mean temperature (°C), daily minimum and maximum temperature (°C), daily precipitation amount (mm), daily means of relative humidity (%) and surface pressure (hPa). In this study the meteorological data from the nearest grid point to the location of interest were used. The coordinates of the grid point are N46.6, E17.6. The monthly values were calculated based on daily mean temperature (°C), minimum temperature (°C) and precipitation amount (mm) of the year 2020.

2.4. Sampling and experimental setup

Sampling took place on 8–06–2020, when the strawberries were in full ripeness. A total of 30 fruits per sample type were randomly collected and delivered immediately in a cooler to the food physics laboratory of Kaposvár Campus of Hungarian University of Agronomy and Life Sciences in order to set up the refrigeration experiment and perform the investigations. Table 1 contains the information on the names of the samples, their abbreviations, dates of planting and sampling and also the origin of each sample used in the experiment (Table 1.).

The weight, length, width and skin color were measured in an air-conditioned room (at 20 °C). Subsequently, the samples were homogenized and analyzed with spectrophotometer.

Sample processing took place on the day of sampling (8–06–2020), then on the fifth day of refrigeration storage (4 °C) using 10 fruits per each ample type in order to create a homogenous sample from all of sample types.

In addition, a sample of strawberries ('Asia' 2020 Supermarket) purchased from a supermarket was included in the experiment, in order to provide a point of view for comparison among fruits purchased from a local farm and fruit (of Spanish origin) purchased from a multinational company (Table 1.).

2.5. Measurement of weight, length, and width

The weights of the samples were determined on a laboratory bench scale. Length and width values were measured with a digital caliper.

Table 1
Sample information.

Sample name	Sample name abbreviation	Date of planting	Date of sampling	Origin
'Asia' 2018 open field	'Asia' 2018	09–08–2018	08–06–2020	local
'Asia' 2019 open field	'Asia' 2019	08–08–2019	08–06–2020	local
'Asia' 2020 open field	'Asia' 2020	19–03–2020	08–06–2020	local
'Asia' 2020 GH	'Asia' 2020 GH	19–03–2020	08/06/2020	local
'Asia' 2020 Supermarket from Spanish Import	'Asia' 2020 SM	unknown	08–06–2020	imported

2.6. Sample preparation, homogenization, purification

The samples for the measurements were prepared as follows: ten fruits of each samples types were homogenized with a stainless steel hand blender creating a homogenous pulp. The homogenized sample was aliquoted into 2 mL Eppendorf tubes and centrifuged in a refrigerated Hettich, MIKRO 220R (Andreas Hettich GmbH & Co. KG Föhren str., 12 D-78532, Tutingen, Germany) centrifuge for 5 min (10000 g, 4 °C). After centrifugation, the supernatant of the purified samples was transferred to additional 2 mL Eppendorf tubes, from which pH measurements, Brix index determinations, and spectrophotometric analyses were then performed.

2.7. Colour measurement

The color was determined with an X rite chromameter (X-Rite Corporate Headquartes, USA; Grand Rapids, MI 49512 United States). "L", "a" and "b" values were recorded during the measurement. These are called color points, also known as color coordinates, which the equipment represents in a rectangular coordinate system and is used to characterize colors in the CIE color space.

In the a * b * plane, the a * and b * half-axes correspond to the following colors: + a * is red, -a * is green, + b * is yellow, -b * is blue. L * is the luminance factor located perpendicular to the b * plane (Commission International De I 'Eclairage, 1976).

2.8. Measurements of total soluble solids

The total soluble solid content was determined with a digital refractometer, HI96813 (Hanna Instruments, Hanna Instruments Ltd, Eden Way, Pages Industrial Park, Leighton Buzzard, Bedfordshire LU7 4AD). The concentration of solutes in the solution or pulp (total soluble solids, w/w%) was measured based on the refractive index of the samples, which shows a significant correlation with the sugar content in the case of strawberries.

2.9. pH measurments

The pH of the product was determined with a Testo 205 (Testo Ltd, Newman Lane, GU34 2UR Alton Hampshire, United Kingdom) portable pH meter.

2.10. Measurement of lipid oxidation

Malondialdehyde (MDA) content was determined by the thiobarbituric acid (TBA) reaction with some modifications of the original method Heath and Packer, [24]. From the clear supernatant 0.25 mL of 50× diluted strawberry pulp was added to 1 mL of 20% TCA containing 0.5% TBA, gently mixed and briefly centrifuged for 5 s. The solutions were incubated in a water bath (Julabo ED-5M, JULABO GmbH 77960 Seelbach/Germany) for 30 min at 96 °C. The reactions were stopped by cooling the solutions immediately on ice followed by centrifugation at 10000 rpm for 5 min. Absorbance at 532 and 600 nm was recorded using a SmartSpec™ Plus (Bio-Rad Ltd.1000 Alfred Nobel Drive Hercules, California 94547, USA) spectrophotometer, and MDA concentration was calculated by subtracting the non-specific absorption at 600 nm from the absorption at 532 nm using an extinction coefficient, 156 $mM^{-1}cm^{-1}$. The results were expressed as $nmol{\cdot}g^{-1}$ in mL pulp as the averages of three independent measurements.

2.11. Ferric reducing antioxidant power (FRAP) assay

Total antioxidant activity is measured by the modified assay of ferric reducing antioxidant power (FRAP) of Benzie and Strain, [24]. The constituents of the FRAP reagent are the followings: 10 mM sodium-acetate buffer (300 mM pH 3.6), TPTZ (2, 4, 6-tripyridyl-*s*-triazine) in 40 mM HCl and $FeCl_3x6H_2O$ (20 mM). The working FRAP reagent was prepared by mixing acetate buffer, TPTZ and $FeCl_3x6H_2O$ in the ratio of 10:1:1 at the time of use. Standard solution was 1000 μM ascorbic acid prepared freshly at the time of measurement. 0.1 mL of 25× diluted strawberry pulp was mixed with 2.9 mL of working FRAP reagent. After vortexing, the samples were placed in water bath (Julabo ED-5M, JULABO GmbH 77960 Seelbach/Germany) at 37 °C for 4 min, thereafter the absorbance was measured at 593 nm using a SmartSpec™ Plus (Bio-Rad Ltd.1000 Alfred Nobel Drive Hercules, California 94547, USA) spectrophotometer. Ascorbic acid standards (100 μM–1000 μM) were processed in the same way. Results were calculated in ascorbic acid equivalents, as the averages of three independent measurements.

2.12. Ultra-weak bioluminescence measurement

A CCD camera-equipped NightShade LB 985 (Berthold Technologies, Bad Wildbad, Germany) plant imaging system), controlled by IndiGO™ software was used to detect bioluminescence. Since illumination provides excitation for the electronically excitable radicals, which then fall into their base state by emitting 50 times higher light intensity, compared to non-illuminated samples [12,25]; fruits were illuminated with LED panels: far red (730 nm) with 2, red (660 nm) with 105, green (565 nm) 40, and blue (470 nm) 110 mol m^{-2} s^{-1} for 5 s after their placement into the dark chamber and prior to the initiation of bioluminescence measurement. In order to decrease the dark current, the camera was cooled to −68 °C by the controller software of the NightShade LB 985 apparatus. After turning off the LEDs, luminescence was monitored for 5 min and the recorded photon counts were visualized by IndiGO™ software and converted to counts per second (cps) values, then normalized for area (mm^2). Five samples were measured per each sample type and

the average and standard deviations are presented.

2.13. Statistical analysis

The effect of time was analyzed with Two-Sample T probe and the effect of sample type was analyzed with one-way ANOVA combined with Duncan post-hoc test by SPSS 20.0 and R-4.3.0 statistical programs.

The Shapiro-Wilks normality test was used to prove the applicability of ANOVA, accordingly if the p-value exceeded the 0.05, the dataset could be considered normally distributed. The p-values for the parameters of colour measurements (L*value, a*value, b*value) were $p_L = 0.1439$, $p_a = 0.1023$ and $p_b = 0.4268$, respectively. The other initial condition for ANOVA is the fulfilment of the Homogenity of Variances, which was calculated by Bartlett test. The p-values were $p_{[Sample \times L^*value]} = 0.3187$, $p_{[Sample \times a^*value]} = 0.3537$, $p_{[Sample \times b^*value]} = 0.4161$, respectively, so the null hypothesis, i.e. the group variances supposed to be equal, could not be rejected.

3. Results

3.1. Meteorological background in 2020

The weather in 2020 was unfavorable for horticultural plant production in Hungary. The first five months of the year were characterized by drought associated with unexpected cold outbreaks of the year were characterized. The monthly sums of precipitation can be seen in Table 1. Until the end of May, only 140 mm of precipitation had been detected. However, the main damaging factor for strawberry production is macro-scale cold advection below zero degrees Celsius in early spring. In 2020, due to the higher than average temperatures in February and March (Table 2), the growing season could start earlier. Flower buds and flowers had already appeared on the plants when the first wave of Arctic airmasses arrived in the Carpathian basin on 1st of April causing extensive frost damage. In the location of interest, the lowest temperature on 1st April was −3.77 °C, 2nd April −5.46 °C, 3rd April −2.44 °C. The latest frost happened on 15th April (−1.39 °C) (Table 3). 33% of the flower buds and flowers of strawberry plants suffered frost damage. In June, the temperature was higher than average accompanied with high intensity convective systems, so the warmer and wetter conditions were favorable for rot of the fruits.

3.2. External properties and content-related investigations

3.2.1. Appearance-, weight- and size-related parameters

The results of the weight measurements in Fig. 1 show the total weights of the samples taken on the day of sampling for the different varieties.

The average weight of the sample group from the ‘Asia’ Supermarket (SM) was significantly higher than the average weight of the other varieties, however, the large standard deviations imply lack of uniformity (Fig. 1A). In addition, the total weights of the ‘Asia’ 2019 and 2020 samples were also significantly higher than the 2018 samples and the 2020 GH sample, respectively (Fig. 1A). The statistical analysis of the sample weights indicated that the investigated samples created three statistically different groups. The first group consisted of ‘Asia’ 2020 GH with the lowest weight values, whereas ‘Asia’ 2019 and ‘Asia’ 2020 belonged to the second group; and ‘Asia’ SM was the third group with the highest weight values of all. Similar grouping patterns were observable in the results of width and length (Fig. 1B–C). Both latter parameters and weight had the highest values for ‘Asia’ SM. Length data, similar to the results of the weight measurement, showed that the values of the oldest, 2018 plantation had the lowest weight of all. Based on the statistical analysis, the berries in the ‘Asia’ SM sample were significantly longer than similar values in the other samples. A similar trend was observed in the width measurement results (Fig. 1B–C).

3.2.2. Changes of colour of strawberry samples

The results of the color changes are presented in Figs. 2 and 3. The color measurements (Fig. 3A–I.) show a decreasing tendency for the redness (Fig. 3A,D,G.), yellowness (Fig. 3B,E,H) and also for the lightness (Fig. 3C,F,I.) of strawberry samples after five days of 4 °C storage.

Differences in color parameters among sample types. The statistical analysis of the sample types within the sampling times revealed that redness, differed significantly ($p = 2.35 \times 10^{-2}$) among the samples on the first sampling day (Fig. 3A.). However, no significant color differences for yellowness on the first ($p = 6.15 \times 10^{-3}$) (Fig. 3B.) and fifth ($p = 1.01 \times 10^{-1}$) sampling day (Fig. 3E.). The lightness of the samples belonged to the same statistical group on the first day ($p = 7.96 \times 10^{-1}$), but after 5 days of storage, ‘Asia’ (2018, 2019) has significantly lower lightness values ($p = 5.47 \times 10^{-5}$) compared to ‘Asia’ (SM) (Fig. 3C,F,I). For all color parameters, ’Asia’ SM showed the lowest values.

Table 2
Monthly sum of precipitation in the vegetation period of strawberry in 2020.

	January	February	March	April	May
Monthly precipitation	20.1 mm	31.3 mm	33.5 mm	17.7 mm	82.1 mm

Table 3
Changes of TSS, pH and dry matter content of strawberry samples during five days of storage.

Sample	*Days*	*Total soluble solids (w/w%)*	pH	*Dry matter content (%)*
'Asia' 2018	1. day	9.6	3.61	12
	5. day	13.5	3.64	17
'Asia' 2019	1. day	9.5	3.72	12
	5. day	10.7	3.81	13
'Asia' 2020	1. day	9.7	3.70	14
	5. day	12.5	3.74	16
'Asia' 2020 GH	1. day	9.5	3.94	11
	5. day	10.1	4.00	12
'Asia' SM	1. day	6.4	3.00	8
	5. day	6.3	3.64	9

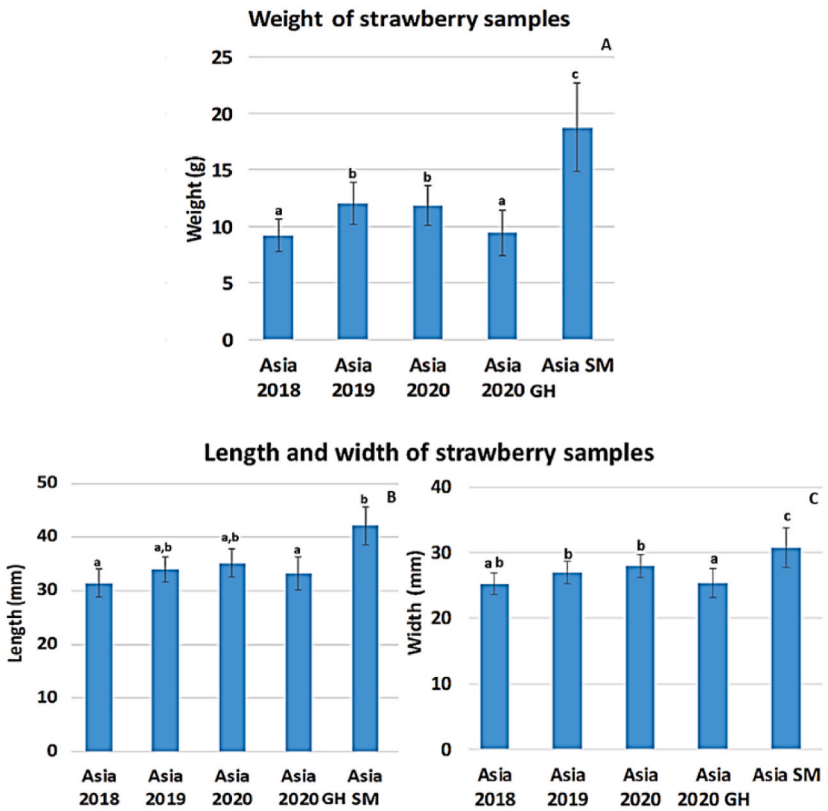


Fig. 1. Weight (g), length and width values (mm) of different strawberry samples measured with a caliper on the day of sampling. Different letters indicate significant differences between samples ($p < 0.05$).

Fig. 2. Strawberry samples on the 1st and on the 5th day of storage.

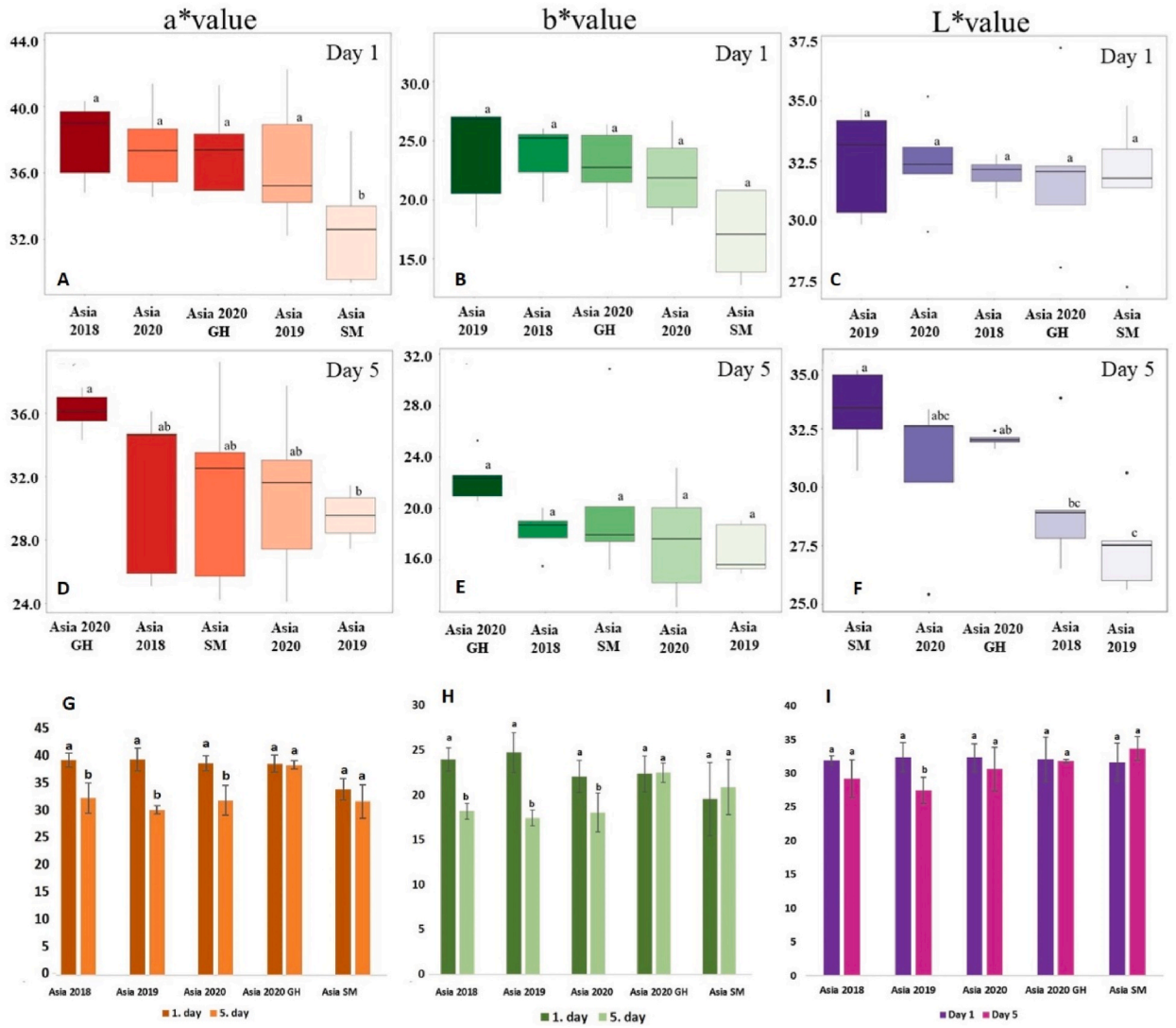


Fig. 3. Changes of color intensity (redness: a * (**A,D,G**), yellowness: b * (**B,E,H**), lightness: L * (**C,F,I**) color coordinates) during storage among sample types of Day 1 (**A, B,C**) and Day 5 (**D,E,F**) and between sampling occaions (**C**). Different letters indicate significant differences ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Differences in color parameters induced by storage. The results of the Duncan test show (Fig. 3 G,H,I.) that redness decreased significantly in 'Asia's 2018; 'Asia's 2019; and 'Asia's 2020), whereas although the decrease was observable in all of the samples, it was not significant in 'Asia' 2020 GH and 'Asia' 2020 SM (Fig. 3G.). The most pronounced color parameter change occurred in the case of the yellowness of the samples. It decreased significantly by approximately one-fourth of the initial value in 'Asia in 2018, 'Asia' in 2019, and 'Asia' in 2020. In the case of 'Asia' 2020 GH and 'Asia' SM, the yellowness did not change significantly, however the average value even showed a slight increase in the case of 'Asia' SM (Fig. 3H.). The lightness of strawberry samples showed similar changes to yellowness, although the rate of decrease was not that pronounced and only decreased significantly in the case of 'Asia" in 2019 after 5 days of storage (Fig. 3I.).

3.3. Measurements of the processed strawberry samples

3.3.1. Results of the pH and total soulbe solids measurement of strawberry homogenate

On the day of sampling and after the samples had been kept for five days, the pH and TSS were measured. The TSS of the samples of local origin were similar: 9.6; 9.5; 9.7; and 9.5 for 'Asia' in 2018; 'Asia" in 2019; 'Asia' in 2020; and 'Asia' GH 2020, respectively, whereas the TSS of 'Asia' SM was lower: 6.4. The greatest increase was observed after five days of storage in 'Asia' (2018): 24.3%. The increase in TSS for 'Asia' 2020 is 22.4%; for 'Asia' 2019, it is 11.0%, and for 'Asia' 2020, it is 5.9%. The only sample in which a decrease was found was 'Asia' (SM: 1.19.7; and 9.5 for 'Asia' in 2018; 'Asia' in 2019; 'Asia' in 2020; and 'Asia' GH 2020, respectively, whereas

the TSS of 'Asia' SM was lower: 6.4. The greatest increase was observed after five days of storage in Asia (2018): 24.3%. The increase of the TSS of'Asia' 2020: 22.4%; 'Asia' 2019: 11.0% and 'Asia' 2020 GH: 5.9%. The only sample in which a decrease was found was Asia (SM: 1.1%) (Table 3). The initial pH values in the local samples ranged from 3.61 to 3.94 (Table 3), but the supermarket sample had the lowest pH value: 3.00. As for the changes of pH during the storage period, all of the tested samples increased in pH; the greatest increase was in the case of 'Asia' (2019): 0.9, whereas the highest pH value after storage was in the case of 'Asia' (2020): 4.0. The dry matter content of a 1 g fresh sample differed among the samples. 'Asia' 2020 had the highest dry matter content, and 'Asia' SM had the lowest. The dry matter content of all the investigated strawberry samples degraded during the 5-day storage period. The dry weight of 'Asia in 2018 increased the most (Table 3).

3.3.2. Ferric reducing antioxidant capacity and lipid oxidation measurements of strawberry pulp

The results of the antioxidant capacity (Fig. 4A–C.) and lipid oxidation (Fig. 5A–C.) were looked at from two different points of view. The differences came from the different types of samples that were tested and how long they were stored.

3.3.2.1. Differences in antioxidant capacity among sample types. There were considerable differences among the investigated sample types on the day of sampling. 'Asia in 2019 has the highest, statistically different FRAP value, followed by 'Asia in 2018 and 'Asia in 2020, the three latter being in the same statistical group. 'Asia' SM had the lowest FRAP value, being significantly lower than all of the other samples with the exception of 'Asia GH 2020. After 5 days of storage, the tendencies of differences remained the same for all of the samples, except that the distinction was more statistically defined (Fig. 4A and B.).

3.3.2.2. Differences in antioxidant capacity induced by storage. 5 days of storage resulted in a significant decrease in FRAP values in all of the investigated samples. After five days of storage, 'Asia' 2019 had the highest FRAP value and 'Asia' SM had the lowest. 'Asia' 2020 GH and 'Asia 2020 SM samples showed the greatest decreases, 44 and 46%, respectively. The FRAP value of 'Asia' samples from

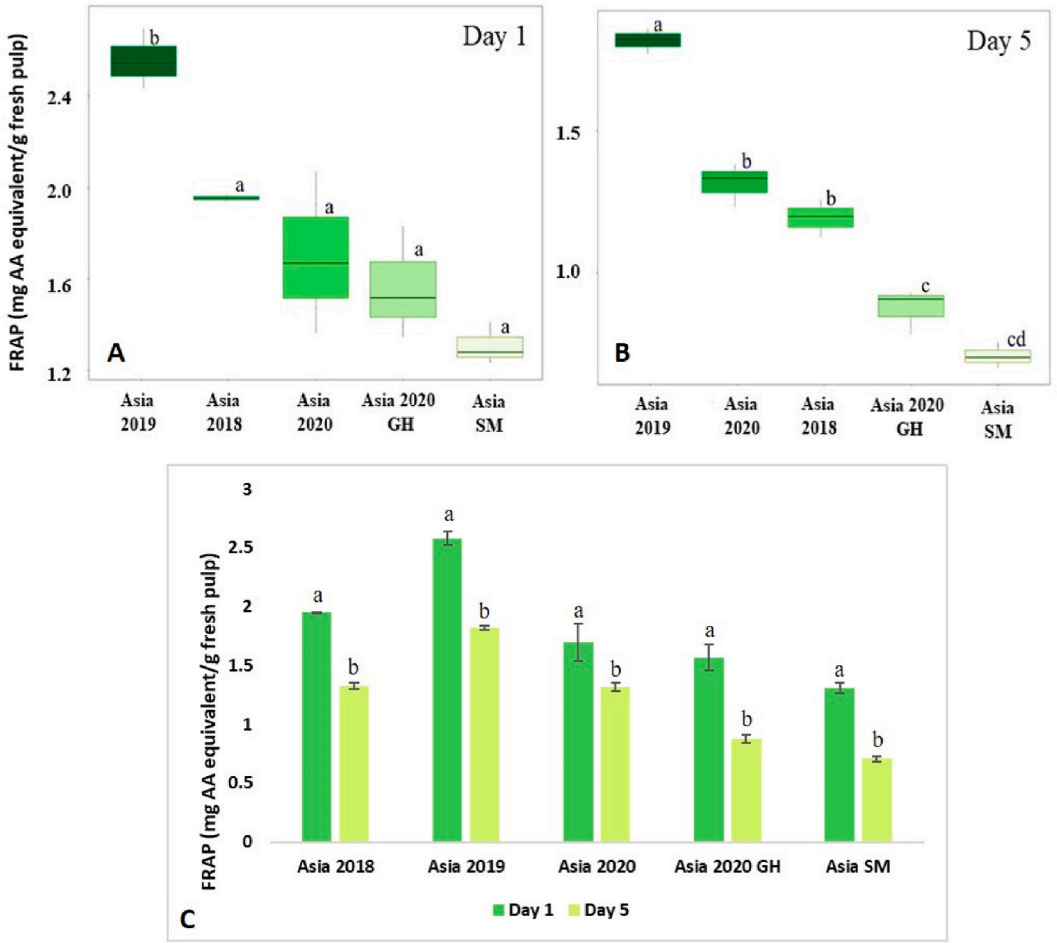


Fig. 4. Changes of antioxidant capacity (FRAP) level of strawberry samples during storage among sample types of day 1 (**A**) and Day 5 (**B**) and between sampling occaions (**C**). Different letters indicate significant differences ($p < 0.05$).

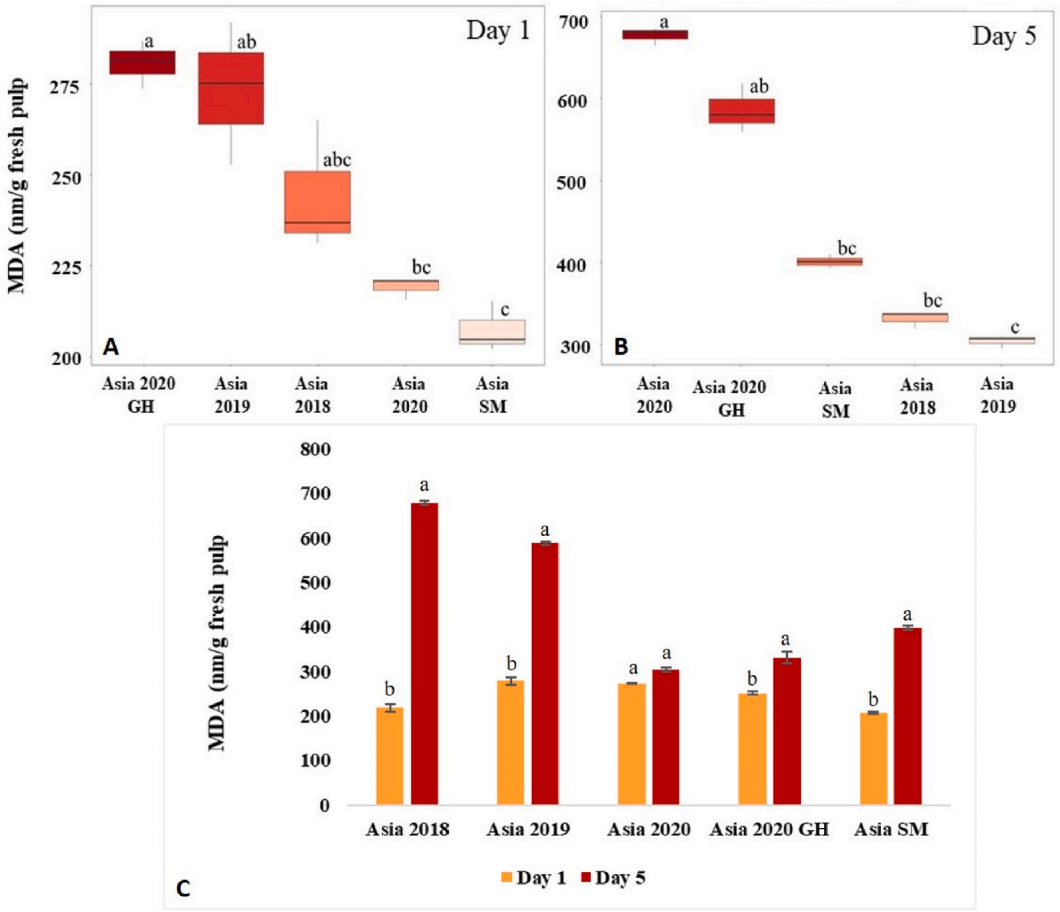


Fig. 5. Changes of lipid oxidation (MDA) level of strawberry samples during storage among sample types of day 1 (**A**) and Day 5 (**B**) and between sampling occaions (**C**). Different letters indicate significant differences ($p < 0.05$).

2018 cultivation decreased by 34% and 33sulted in a significant decrease in FRAP values in all of the investigated samples. After five days of storage, 'Asia' 2019 had the highest FRAP value and 'Asia' SM had the lowest. Asia 2020 GH and Asia 2020 SM samples showed the greatest decreases, 44 and 46%, respectively. The FRAP value of Asia samples from 2018 cultivation decreased by 34% and 33%, respectively, and the lowest decrease occurred in 'Asia in 2019 and 2020 by 30.7% and 17.6ve days of storage, 'Asia' 2019 had the highest FRAP value and 'Asia' SM had the lowest. Asia 2020 GH and Asia 2020 SM samples showed the greatest decreases, 44 and 46%, respectively. The FRAP value of Asia samples from 2018 cultivation decreased by 34% and 33%, respectively, and the lowest decrease occurred in Asia in 2019 and 2020 by 30.7% and 17.6%, respectively (Fig. 4C).

3.3.2.3. Differences of lipid oxidation among sample types. 'Asia' 2020 showed the highest and statistically different lipid oxidation rate compared to the other samples. 'Asia's 2018 and 2019 MDA values were significantly lower, followed by 'Asia's 2020 MDA values. The MDA level was the lowest in the case of 'Asia' SM (Fig. 5A and B). Compared with FRAP values, an opposite pattern and tendency of lipid oxidation was observed in the case of samples obtained from plants planted in 2018 and 2019.

3.3.2.4. Differences of antioxidant capacity induced by storage. Storage increased the MDA values of all investigated samples significantly, except for 'Asia' 2020. The changes in lipid oxidation were pronounced: 310, 244, 192, 131, and 111% for 'Asia" in 2018, 'Asia" in SM, 'Asia" in 2019, 'Asia" in 2020 GH, and 'Asia" in 2020, respectively, after five days of storage (Fig. 5C).

3.3.3. Bioluminescence measurements

In addition to the spectrophotometric examination of the lipid oxidation level, we also performed ultra-weak luminescence-based biophoton emission tests on the samples by illuminating them with a white light in order to excite the excitable compounds and species of the sample, the photon emission rate was measured every minute for 5 min. The image shows (Fig. 6) the biophoton emission values of each strawberry sample over a 5-min measurement period. Each image represents the changes in the biophoton emission values per minute during the 5-min measurement period. Taking into account the highest cps values of the images represented by the red

pseudocolor per image (Fig. 6), the results show that the Asia 2018 treatment had the highest initial biophoton emission value (7289 cps) (Fig. 6A.), followed by Asia 2019 (4800 cps) (Fig. 6F.), and Asia SM (3041 cps) (Fig. 6U.). The lowest values were for the Asia 2020 GH (977 cps) (Fig. 6O.) and Asia 2020 (885 cps) (Fig. 6K.) treatments. We also analyzed the samples statistically and discovered statistically significant differences among all treatments.

However, it can be seen from the presented figure that the initial value alone does not give a complete picture of the fruit's biophoton emission; therefore, in the following figure, the total biophoton signals emitted over the 5 min were summed (Fig. 7A and 7B.) and presented in order to approximate a more precise overall hint about the quality of strawberry samples. In this case, the data was analyzed and normalized for the unit of area (mm^2). The results of the photon summary indicate similar but parametrized differences among the samples that were further confirmed by the results of the decay kinetics of photon emission (Fig. 7A). Furthermore, the biphoton results were similar to those of the MDA results after five days of storage (Fig. 5B and C.).

In terms of biophoton emission, the initial values on the one hand and the dynamics of the decay on the other hand can provide information on the quality of the fruits. It can be deduced that the treatment with the longest period of photon emission decay is considered to be the treatment with the highest initial value (Fig. 7B.), and the summed biophoton emission values of each treatment are significantly different from each other for each treatment, with the only exceptions being Asia 2020 and Asia 2020 GH. The preceding results were supported by visual representations of the lipid oxidation process and the quantitative parameters derived from these results: first the total signal emission during 5 min (Fig. 7A); and, to clarify the preceding results and establish the decay dynamics, we plotted the photon emission results during 5 min (Fig. 7B). These data indicate that the samples with the highest initial photon emission and the longest decay time were the ones whose analytical results demonstrated the greatest quality degradation during storage.

The results are congruent with those of the malondialdehyde investigation, which found that the Asia 2018 sample had the maximum intensity of the biophoton emission per unit area of the signal, similar to what was observed in the analytical lipid oxidation measurement. For the remaining samples, the trends mirrored those of the analytical measurement.

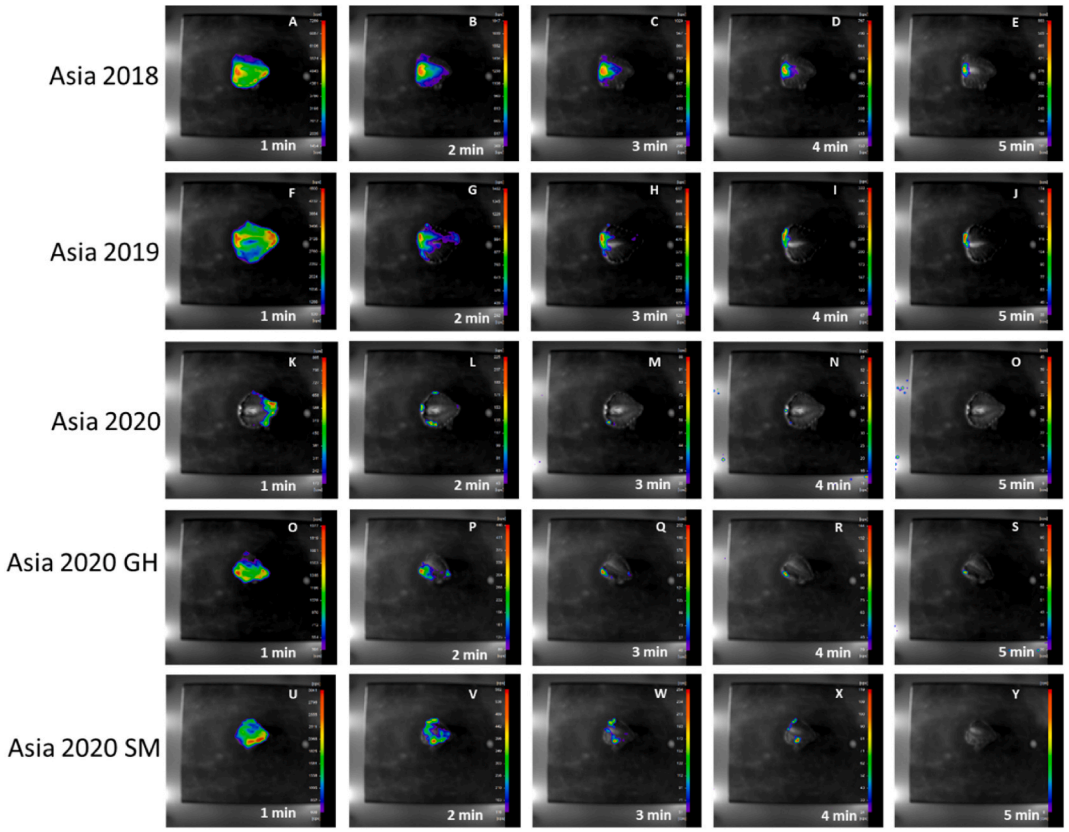


Fig. 6. Images of ultra-weak bioluminescence intensities, as a function of time, after 5 days of houshold refrigeration storage. Images were taken by the CCD camera-equipped NightShade LB 985 plant imaging system and analyzed by IndiGO™ software that associated a pesudo-colour scale to the emitted photon amount and assigned quantified (cps) values to the detected photon intensities that are shown in the right side of each image (**A-X**), otherwise no numerical value is displayed, such as for Asia 2020 SM sample in the 5th minute of the measurement (**Y**). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

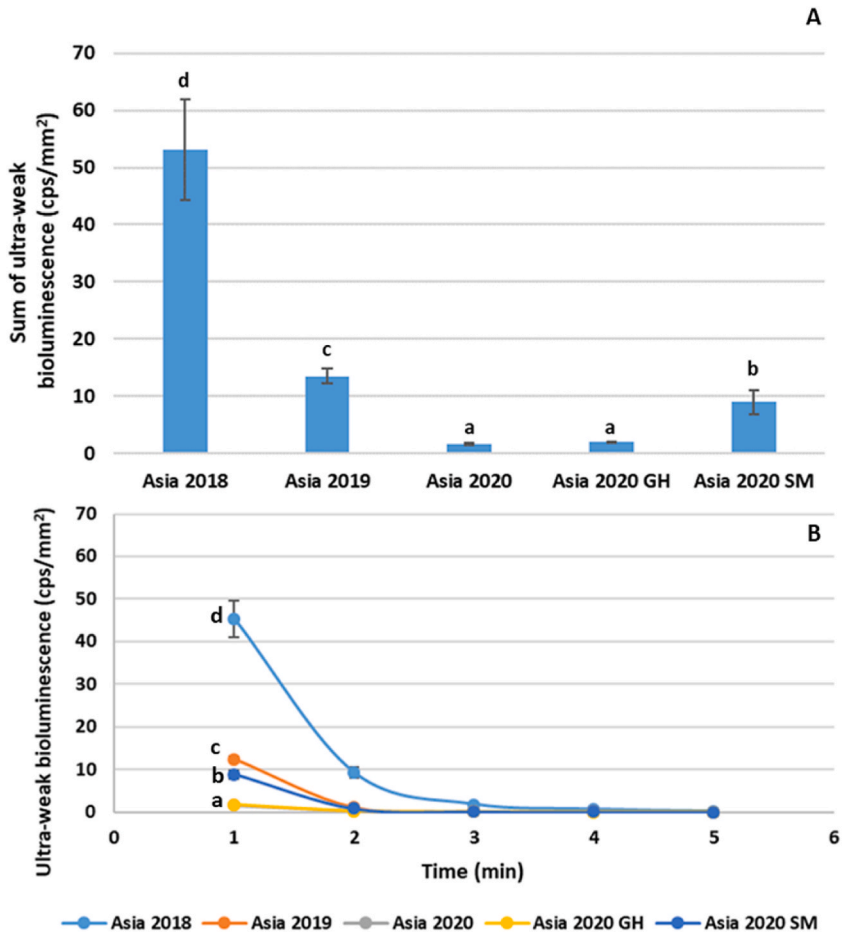


Fig. 7. **(A)** Sum and the decay **(B)** of ultra-weak bioluminescence intensities after 5 days of houshold refrigeration storage.

4. Discussion

In this work, we aimed to characterize the quality changes of differentially grown strawberry samples subjected to five days of 4 °C storage in order to provide a model for home refrigeration before the consumption of the fruit. Furthermore, the presented experimental setup enabled not only the evaluation of the storage-induced quality changes of strawberries but also a distinction of age and sample types.

Differences among sample types: the samples from 2018 gave the worst overall impression, since the decrease in yellowness was the most pronounced, along with their weight and lipid oxidation being significantly lower compared to samples from 2019 to 2020 that indicates an age effect on fruit mass and quality. The more years a strawberry plant spends in production, the more likely the tendency for smaller fruit size is, which leads to a decrease in performance. Radajewska and Dejwor [26], studying six different varieties of strawberries. Based on their results, the average yield decreased significantly in the third year of cultivation, which was due to a combined decrease in size and weight in addition to the number of fruits [27]. Moreover, the meteorological data revealed that the growing season of 2020 started with a mid-spring frost that might have affected the overall yield and resulted in a reduction in the size of local fruits. Moreover, 'Asia' 2020 GH samples had significantly less weight compared to the other samples from the 2020 plantation, the reason for which may lie in the cultivation system. One of the advantages of growing strawberries in a greenhouse is the enhancement of earliness [26], as a result of which the greenhouse-grown strawberry sample was at a more advanced phenological stage, as the fruits ripen earlier than in the open field. The weight results of the samples from the supermarket may be the result of different factors in this case as well. In Spain, there are two types of one-year planting structures: macro- and microtunnels [28]. This means that the first-year fruits are harvested, when they are the greatest both in size and weight. Furthermore, fruits also undergo a sorting process before packaging; all these factors contribute to the high measured weight values for supermarket samples. In addition, the statistical evaluation also highlighted differences among sample types. The FRAP value of the supermarket sample was the lowest, due to early harvest and subsequent handling technology. Further, low dry matter content was observed as well, which consequently means lower non-enzymatic antioxidants, as depicted through the changes in FRAP values. In contrast, the'Asia' 2019 sample had the highest antioxidant capacity, and all the local fruit had higher antioxidant capacity.

Differences induced by storage: during the five days of storage, pH changed towards the neutral direction, indicating a decrease in acid content during storage. This is in accordance with the results of Paranpje et al. [27] and Koyuncu and Dilmaçünal [29], who documented the reduction of citric acid, malic acid, oxaloacetic acid, succinic acid, and, to the greatest extent, ascorbic acid. The low initial value of the 'Asia' SM sample probably arose from product manipulation before storage and shipping, since according to the results of Dhital et al. [30], limonene liposome-treated strawberries had significantly lower pH values as compared to control. This implies a similar process for our study as well, despite the fact that the nature of pretreatment remains unknown. Moreover, this sample may have been harvested at an earlier ripening stage with more acidic content in order to increase its shelf life [31], which induces inferior quality traits in terms of taste, color, and texture [32], as our results confirmed.

The color of ripe, fresh strawberries is bright red, and during cold storage, the color constantly changes [33] towards darker hues. Our results are consistent with those of Almenar et al. [2], Caner et al. [7], and Kartal et al. [3], who observed decreasing tendencies in the color coordinates L*, a*, and b*. In our work, in most cases, a significant decrease was observed between the values measured on the day of sampling and after five days of storage. However, the color did not change for the "Asia' SM sample, as a possible result of the color-preserving effect of manufacturing and packaging technologies, such as those described in the work of Ikegaya et al. [34], where color did not change during 28 days of storage at 0 and 3 °C in prepackaged strawberries covered with freshness-preserving film. Also, Zhao et al. [35] investigated a package system of an insulated expanded polystyrene box installed with phase change materials equipped with a modified atmosphere package [35] and found a significant effect on maintaining firmness, color, total soluble solids, global appearance, and the respiration rate of strawberries (Fig. 6).

Storage also affected the solute content, as total soluble solids increased with time as a consequence of water loss and lipid oxidation (Fig. 4B) by respiration; moreover, according to Ayub et al. [36], this was a possible result of the breakdown of complex sugars, e.g., polysaccharides and oligosaccharides, into simpler soluble sugars, e.g., monosaccharides.

As for the antioxidant properties of strawberries, according to our results, storage has a negative effect, since it generally lowers the amount of non-enzymatic antioxidants in strawberries. Wicklund et al. [37] observed a decrease in antioxidant capacity in stored strawberry jam and Galani et al. [20] described a significant decrease in vitamin content and antioxidant capacity in fresh fruits and vegetables, which is consistent with the FRAP value changes we observed.

Furthermore, storage resulted in a significant increase in lipid oxidation in all samples except for 'Asia' 2020. The ratios of changes in the 2018 samples showed a significant increase in lipid oxidation levels during storage, whereas the 2019 and 2020 samples showed a minor increase. This also may be the result of the older plantation, where not only the weight but the quality properties of strawberries decrease with time. The MDA value of the stored 'Asia' 2018 sample was significantly higher than the 'Asia' 2019 and 2020 samples, respectively. The concentration of strawberry malondialdehyde increased during refrigeration storage, which indicates an increase in the rate of degradation originating from respiration processes, similar to the experience of Yang et al. [38]. conducted also under 4 °C storage conditions. Furthermore, Younge et al. [39] found that the rate of respiration increased with storage time and also varied at constant temperature as a result of the handling of the fruit.

Nontheless, since photon emission occurs when oxidative processes as lipid peroxidation create metastable excited states (i.e.: triplet carbonyls and singlet oxygen) [40], therefore a bioluminescence-based study was also conducted on the saples. Our results verified that the quality changes throughout the 5 days of cold storage were similarly reflected in changes of lipid oxidation and UWLE values. In this instance, there is a link between the number of light-excited radicals produced during the process of which release their excitation energy through photon emission, and the intensity of the photon emission [40,41]. These works raise attention to the importance of the composition and maturity rate of fruits and their correlation with UWLE [41], which was confirmed by the analytical approximation in our work.

Our results prove that the intensity of photon emission was similar to that of the lipid oxidation state of the samples, which is a natural process during fruit decay and the intensity of which was possible to follow non-invasively via photon emission. Our results are in line with the work of Sivankalyani et al. [42], who detected the luminescence of peroxidized lipids in avocado fruit. In this work, the increase of UWLE corresponded to the increase of lipoxygenase gene expression, the product of which is the lipoxygenase enzyme that catalyzes membrane lipid peroxidation [42]. However, specific questions, such as the energy-absorbing capacity of excitable compounds [43], are yet to be explored in detail. Moreover, the characterization of healthy and infected samples with adenosine triphosphate-triggered bioluminescence (ATP-BL) method [18] would also claim positive addition to fruit quality measurements. Furthermore, since this technique is not selective for the detection of specific ROS of the process of lipid oxidation, combination of autoluminescence measurement with the usage of fluorescence dyes such as -dichlorofluorescein diacetate for H_2O_2 [44] may aid to deepen this knowledge in the future.

5. Conclusion

As a conclusion of this research, it can be stated that three years' plantation is not only unfavorable for the yield of strawberries but also, due to the high rate of lipid oxidation that is unfavorable for storage properties as well. Five days of 4 °C refrigeration, had negatively altered several measured parameters. Weight, color, pH, Brix index, ferric reducing antioxidant capacity, and lipid oxidation demonstrated diverse features of water loss and parallel degradation processes caused by 5 days of 4 °C refrigerator storage. The 'Asia' SM sample's storage tendencies differed from those of locally manufactured samples, likely due to product tampering to extend fruit shelf life after extensive shipping and storage. The 'Asia' 2020 SM sample had a longer shelf life and kept its color, but its FRAP value was notably the lowest at the conclusion of storage, indicating a significantly lower nutritional value than all local samples.

Overall, this research attempted to call attention to the quality differences between local and imported strawberries that customers encounter at home storage. The results of our work justify the significance of local fruits and provide information for customers,

encouraging them to acquire strawberries from local farms not only for the reason of supporting the local economy but also for a more health-conscious attitude.

Furthermore, the rate of lipid oxidation is in line with the intensity of biophoton signals, indicating that UWLE technique is suitable for the non-invasive characterization of strawberry quality. This could be a promising tool for the development of post-harvest technologies for increasing shelf life. If calibrated and standardized, ultra-weak bioluminescence-based tests are possible to replace labor- and cost-intensive analytical approaches to determine fruit quality.

Author contribution statement

Ildikó Jócsák: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

György Végvári: Analyzed and interpreted the data.

Kristóf Klász: Gabriella Andrássy-Baka: Performed the experiments.

Katalin Somfalvi-Tóth: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Éva Varga-Visi: Performed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

The data is accessible upon request.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This research was funded by the Hungarian National Research, Development and Innovation Office [grant number: 2020-4.1.1-TKP2020] and the New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund [grant number: ÚNKP-20-3-I-KE-7] and Supported by the ÚNKP-20-4-II New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund.". The research was supported by the project 'Preparation for the transition to circular economy in the case of agricultural and green waste' of Environment and Energy Efficiency Operational Programme grant scheme of Ministry of Technology and Industry Hungary under grant no.: KEHOP-3.2.1-15-2021-00037.

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