Scientia Horticulturae 327 (2024) 112843

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Non-destructive and destructive physical measurements as indicators of sugar and organic acid contents in strawberry fruit during ripening

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ARTICLE INFO

Keywords:
Strawberry
Firmness
Ripening index
Primary metabolites
Fruit development

ABSTRACT

Strawberry fruit is appreciated by consumers for its characteristic taste, which can be attributed to the balance between sugars and organic acids content, but strawberries are also a rich source of vitamin C (ascorbic acid). This study focuses on the suitability of different physical parameters, such as ripening index (I_{AD}), firmness and total soluble solids content, as indicators of the content of sugars and organic acids during ripening. Five ripening stages were defined - green, white, ripe red, fully ripe dark red and overripe stage at five different cultivars. As the fruit ripened, the content of ascorbic acid, sugars and sugar/acid ratio increased and the organic acids content decreased, but the changes varied among cultivars. The ripening index (I_{AD}) strongly correlated with the total sugar content (r = -0.72), total organic acids content (r = 0.70) and sugar/acid ratio (r = -0.82). Additionally, firmness correlated well with the content of sugars and organic acids. On the other hand, TSS showed a weaker correlation with the total sugar content (r = 0.38). Although, both the ripening index (I_{AD}) and firmness showed strong correlations with sugars and organic acids content and can help distinguish unripe fruit from ripe fruit, the use of these parameters for distinguishing between different of ripe fruit (from early ripe to overripe) can be limited only to certain cultivars.

1. Introduction

Strawberries are the most commonly grown berry fruit in Slovenia with more than 2 000 tons produced in 2021 (FAOSTAT Crops and Livestock Products, 2021). Among the most popular cultivars in Slovenia but also in other parts of southern Europe belongs 'Clery', but other cultivars such as 'Asia' or 'Aprica' are available on the market. Rapid non-destructive and destructive physical measurements are often used by producers to assess the ripening stage and quality of fruits. The ripening index (I_{AD}), firmness and total soluble solids (TSS) are some of such rapid measures that can be performed to evaluate the ripening stage of the strawberry fruit and can serve as a guide for the producers to pick and sort the fruit at harvest. While firmness and TSS are established methods, the ripening index (I_{AD}), as a non-destructive absorbance method, has not yet been studied in strawberry fruit. However, the I_{AD} has been previously tested on other fruits (Bonora et al., 2014; Infante et al., 2011; Peifer et al., 2018; Ribera-Fonseca et al., 2016; Sjöstrand et al., 2024; Smrke et al., 2023; Ziosi et al., 2008) and has shown potential as useful measure to assess the ripeness of fruit.

The quality and ripeness of strawberries can be indicated by the content of metabolites, such as sugars and organic acids, which also contribute to the fruit's taste and consumer acceptance (Zheng et al., 2019). The major sugars present in strawberries are fructose, glucose and sucrose, and the major organic acids are citric and malic (Kallio et al., 2000). To achieve a good flavor, high content of sugars and a relatively high content of organic acids are needed (Kader, 1991). Organic acids are also involved in the stabilization of the color of the fruit (Pistón et al., 2017). This makes the content of sugars and organic acids important quality parameters for identifying a suitable cultivar and optimal ripening stage. Additionally, strawberries contain a high amount of ascorbic acid, which is recognised as an essential hydrophilic micronutrient, and this makes them an important source of this vitamin for human nutrition (Giampieri et al., 2012). Although high ascorbic acid content would be desirable due to its nutritional value, high ascorbic acid content can contribute to the color instability of processed products from strawberries (Murray et al., 2023). This makes ascorbic

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https://doi.org/10.1016/j.scienta.2024.112843
Received 26 October 2023; Received in revised form 13 December 2023; Accepted 2 January 2024
Available online 9 January 2024

acid content an important parameter especially when choosing the right raw material for processing.

The content of these metabolites changes during their ripening, and understanding these changes is essential for identifying the optimal ripening degree of strawberries at harvest. During the ripening, the fruit softens as the cell wall degrades, and it is highly correlated with the cultivar and preharvest environment (Kader, 1991). Moreover, soluble solids content (SSC) increases during strawberry development, but on the other hand, total acidity (TA) declines during ripening (Montero et al., 1996). The changes during ripening can cause tissue stress and require antioxidant action to prevent cell damage, which can be facilitated by ascorbic acid (Barata-Soares et al., 2004). Therefore, it would be natural to assume that the ascorbic acid content would decrease during ripening. However, it has been reported that the ascorbic acid content increases during ripening (Cruz-Rus et al., 2011; Fecka et al., 2021). Many studies have focused on the changes in the content of sugars, ascorbic acid and other organic acids during the ripening (Fecka et al., 2021; Hwang et al., 2019; Kafkas et al., 2007; Moing et al., 2001; Ornelas-Paz et al., 2013; Pistón et al., 2017; Sturm et al., 2003; Vandendriessche et al., 2013), but a comparison of these changes with the rapid physical non-destructive measurements is needed to identify the most reliable indicators of ripening that can be used by the producers.

The aim of this study was to determine the suitability of different non-destructive (I_{AD}) and destructive physical measurements (firmness and TSS) that indicate the ripening stage and the content of sugars and organic acids. Furthermore, the results of this study describe the change in the content of individual organic acids and sugars during the ripening in the chosen cultivars. This study included five June-bearing cultivars (′Asia′, ′Clery′, ′Aprica′, ′CIVIN 766′ and ′Malwina′) sorted into five ripening stages, as follows: green fruit, white fruit, ripe red, fully ripe dark red and overripe fruit. The fruit of each cultivar was collected over the peak harvest period of each cultivar within one harvest season during 2021. Additionally, based on these results, an optimal ripening stage for harvesting the fruit for consumption and processing can be determined.

2. Materials and methods

2.1. Plant material

Strawberry samples were obtained from five economically important strawberry cultivars: ’Asia’, ’CIVN 766′, ’Aprica’, ’Clery’ and ’Malwina’. Selected cultivars have their cultivar-specific fruit color; light red, almost orange in the case of ’CIVN 766′, light red in cultivar ’Clery’, red in ’Asia’ and ’Aprica’ and dark red in the case of ’Malwina’ cultivar.

Strawberry plants of selected varieties were planted on 2nd September 2019 using green freshly cut runners, except for cultivar ’Asia’, which was planted as frigo plants. The strawberries were produced in accordance with integrated production guidelines. All fruit samples were collected at the same orchard, at the Research Station of the Agricultural Institute of Slovenia located at Brdo pri Lukovici (latitude, 46°10′ N; longitude, 14° 41′ E). Individual cultivars were planted in five blocks, with 10 plants in each block (together 50 plants of each cultivar). The field trial was carried out on soil rich in potassium and nitrogen and low in phosphorus, equipped with a drip irrigation system. Soil texture in the orchard was silty loam with a pH value of 6.1 and mineral composition of 9.5 mg 100 g^{-1} P_2O_5, 27 mg 100 g^{-1} K_2O and 7.8% C organic matter stock. Plants were planted on slightly raised beds covered with black polyethylene. They were arranged in double rows, with 0.25 × 0.25 m spacing between the plants on the bed and 1.3 m spacing between the adjacent beds. Average day and night temperatures in the tunnel ranged between 8.5 °C and 22.1 °C during the production period. Light levels (0–643.2 W m^{-2}) and humidity (63.2–96.4%) were under ambient spring conditions. The experiment was covered with a high polyethylene tunnel from the beginning of flowering (5th May 2021) until the end of harvesting (end of June 2021). This practice represents classic strawberry production technology as the tunnel prevents the rain from damaging the fruit.

Fruit samples were collected during the peak ripening time as defined in Table 1. Each strawberry cultivar was harvested in five different fruit maturity stages during the ripening time: green fruit (R1), white fruit (R2), ripe red fruit (R3), fully ripe dark red fruit (R4) and overripe fruit (R5) as shown on the example of ′Aprica′ cultivar (Fig. 1). Pictures of fruit of other cultivars can be found in Supplementary Material S1. The samples were immediately transported to the laboratory in the Department of Agronomy of the Biotechnical Faculty of the University of Ljubljana (Slovenia), where they were further analysed.

2.2. Postharvest parameters

From each cultivar and each ripening stage, 14 berries were picked. For each berry, the ripening index (I_{AD}) was measured by DA meter FRM01F (Sintéleia, Bologna, Italy), which detected the chlorophyll content in the fruit using absorbance and was previously used as a rapid non-destructive method on strawberries (Simkova et al., 2023). The total soluble solids content (TSS) was measured using the MA885 Wine Refractometer (Milwaukee, USA), and the firmness of the fruit was measured using a digital penetrometer (TR, Turoni, Italy) with a 3 mm diameter probe. In total for each parameter, 14 repetitions were obtained.

2.3. Dry matter

From each sample (each ripening stage of each cultivar), dry matter content was determined by drying the sample in the oven at 105 °C for 72 h The measurement was performed in 6 replicates for each sample type and replicates were prepared by pooling at least 7 fruits. The results were used for recalculating the contents per dry weight.

2.4. Sample preparation

For each extraction, 6 repetitions were prepared and pooled from 14 berries in order to ensure representative results. Samples for sugars and organic acids extraction were frozen in liquid nitrogen and stored at −20 °C until further analysis.

2.5. Ascorbic acid extraction and determination

Ascorbic acid extraction and analysis were performed as described by Simkova et al. (2023). Extraction was done from fresh samples, and sample extracts were stored at −20 °C until analysed by high-performance liquid chromatography (HPLC). Ascorbic acid was analysed by Vanquish HPLC (ThermoScientific, USA) using the column (Rezex ROA-Organic acid $H + 8\%$ (150 mm x 7.8 mm), Phenomenex, California, USA) at the same conditions as described by Simkova et al. (2023). The results are expressed in mg g^{-1} dry weight.

2.6. Sugars and organic acids extraction and determination

The extraction of organic acids and sugars followed the method previously described by Simkova et al. (2023).

Table 1
Overview of the beginning and end of harvest and sampling period during the peak harvest time for each cultivar.

Cultivar	Beginning of ripening/end of harvest	Sampling period
′Aprica′	3.6./30.6.2021	10. - 25.6.2021
′Asia′	5.6./30.6.2021	10. - 25.6.2021
′CIVN766′	28.5./19.6.2021	5. - 20.6.2021
′Clery′	28.5./19.6.2021	5. - 20.6.2021
′Malwina′	10.6./2.7.2021	15. - 30.6.2021

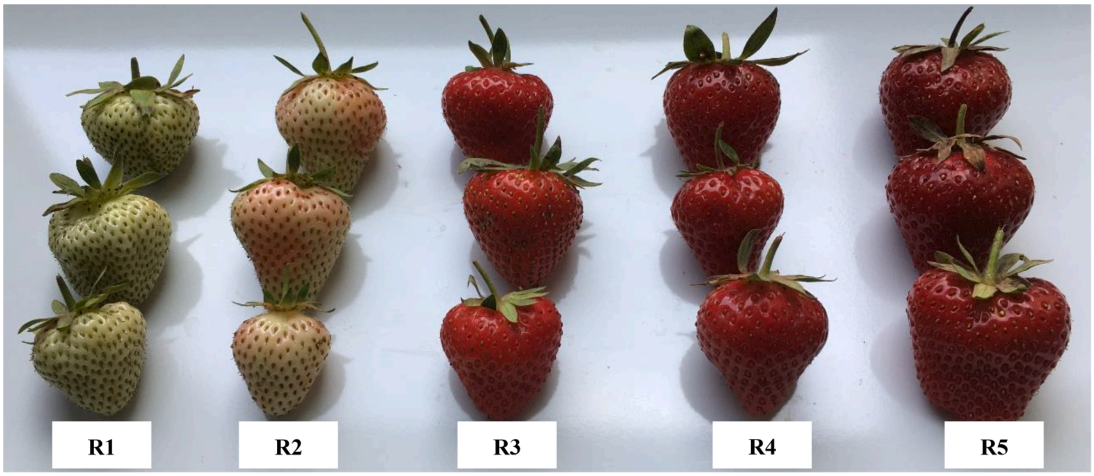

Fig. 1. Five ripening stages of the fruit of the cultivar 'Aprica' (green fruit - R1, white fruit - R2, ripe red fruit - R3, fully ripe dark red fruit - R4 and overripe fruit - R5).

Organic acids were analysed by Vanquish HPLC (ThermoScientific, USA) using the column (Rezex ROA-Organic acid $H+$ 8% (150 mm x 7.8 mm), Phenomenex, California, USA) with 4 mM solution of sulphuric acid in bi-distilled water as mobile phase. Individual sugars were analysed by Vanquish HPLC (ThermoScientific, USA) using the column (Rezex RCM-monosaccharide Ca+ 2% (300 mm x 7.8 mm), Phenomenex, California, USA) with bi-distilled water as mobile phase. All other conditions for the HPLC analysis and quantification of sugars and organic acids were the same as described by Simkova et al. (2023). The results are expressed in mg g^{-1} dry weight.

2.7. Statistical analysis

The data were analysed statistically in R commander x64 4.1.2. The data were expressed as means ± standard error. Significant differences among the ripening stages within each cultivar were determined by one-way analysis of variance (ANOVA) with Duncan's test. Multiway ANOVA was performed to evaluate the effect of cultivar, cultivar and their interaction on the results. Pearson correlation was used to assess the relation of the different parameters with each other. A significant difference, effect or correlation was considered at $p < 0.05$.

3. Results

3.1. Ripening index, firmness and total soluble solid

The ripening index (I_{AD}) decreased with the ripening stages, and there was a significant difference between the green (R1), white (R2) and the last three ripening stages (R3-R5) of the fruit (Table 2). However, there was no significant difference between the ripe fruit of the stages R3 to R5 in most cultivars, except for the cultivar 'CIVN766′, where the ripe fruit (R3) showed higher value than the fully ripe and overripe fruit. The fruit of the last three ripening stages showed similar values (0.10 – 0.33) among the cultivars, except for 'Malwina', where ripe fruit showed higher values than in the other cultivars (0.56–0.63).

Similarly, as for the ripening index (I_{AD}), the fruit's firmness decreased with the ripening stages in all cultivars, and fruit of the last three ripening stages (R3-R5) was significantly softer than green and white fruit (R1 and R2). The fruit of last three ripening stages (R3-R5) showed similar firmness values, even among the cultivars, and except for 'Asia' and 'Aprica', the ripe fruit (R3) was significantly firmer than the overripe fruit (R5).

Additionally, the TSS increased during the ripening, and the fruit in the last three ripening stages (R3-R5) had higher TSS content than green fruit (R1). The only exception was the cultivar 'Asia', where the difference between green (R1) and ripe fruit (R3) was not statistically significant. Among all the cultivars, 'Malwina' showed the highest TSS content.

For all three parameters, the effect of cultivar, ripening stages as well as their interaction was significant.

3.2. Ascorbic acid

The ascorbic acid content increased during ripening from green fruit (R1) to the last three ripening stages (R3-R5) in all studied cultivars except 'Malwina' (Table 3). In 'Malwina', the content was significantly higher only in fully ripe fruit (R4) and overripe fruit (R5) compared to the green and white fruit. Additionally, overripe fruit (R5) of the 'Malwina' cultivar showed higher content than ripe and fully ripe fruit (R3 and R4). In the cultivars 'Asia', 'CIVN766′ and 'Clery', all three stages of ripe fruit (R3-R5) show comparable content. In the cultivar 'Aprica', the fully ripe fruit (R4) showed significantly higher content than ripe (R3) and overripe (R5) fruit. Among the cultivars, the 'Aprica' fruit of edible maturity showed the highest mean content (6.42 mg g^{-1}). This cultivar already showed high content in the green fruit (4.52 mg g^{-1}), which was comparable with ripe fruit (R3) of the other cultivars. The largest increase was detected in the cultivar 'Asia', the content more doubled from green to edible maturity stage. Cultivar and ripening stages, as well as their interaction, showed significant effect on the content.

3.3. Organic acids

The total organic acids content, as the sum of contents of citric, malic, shikimic, fumaric and ascorbic acid, decreased during the ripening in all studied cultivars (Table 3). The organic acids content was higher in the green strawberries (R1) compared to the ripe ones (R3-R4) in all cultivars. The highest mean organic acids content was detected in the green fruit (R1) of the 'Aprica' (212.55 mg g^{-1}), but it was comparable to the content the white fruit (R2) of the same cultivar and the green fruit (R1) of the cultivar 'Malwina'. In the last ripening stages (R3-R5), the 'Malwina' fruit showed the highest organic acids content among the cultivars. Within each cultivar, the organic acids content among the last 3 ripening stages (R3-R5) was comparable except for the cultivar 'Aprica' and 'CIVN766′, where the overripe fruit (R5) showed lower organic acids content than the ripe fruit (R3).

Similar differences can also be observed in the citric and malic acid contents, where the content was higher in green fruit compared to the last three ripening stages (R3-R5), with the exception of 'Malwina' fruit, where only the fully ripe fruit (R4) showed significantly lower citric acid content. The citric acid content was highest in the green fruit of the

Table 2
Ripening index, firmness and total soluble solid (TSS) of the studied strawberry cultivars at 5 ripening stages.

Cultivar	Ripening stage	Ripening index (I_{AD})	Firmness (N)	TSS (%)
Aprica	green (R1)	1.16 ± 0.06 a	7.36 ± 0.43 a	5.70 ± 0.25 b
	white (R2)	0.64 ± 0.03 b	4.92 ± 0.48 b	6.64 ± 0.27 b
	ripe (R3)	0.17 ± 0.01 c	1.70 ± 0.21 c	8.25 ± 0.41 a
	fully ripe (R4)	0.12 ± 0.01 c	1.15 ± 0.12 cd	8.61 ± 0.54 a
	overripe (R5)	0.10 ± 0.02 c	0.73 ± 0.10 d	9.04 ± 0.36 a
Asia	green (R1)	1.09 ± 0.05 a	5.81 ± 0.68 a	6.41 ± 0.17 c
	white (R2)	0.63 ± 0.03 b	3.41 ± 0.36 b	6.86 ± 0.33 bc
	ripe (R3)	0.26 ± 0.02 c	1.47 ± 0.22 c	7.40 ± 0.38 abc
	fully ripe (R4)	0.22 ± 0.02 c	1.12 ± 0.10 cd	7.87 ± 0.49 ab
	overripe (R5)	0.21 ± 0.02 c	0.56 ± 0.08 d	8.33 ± 0.52 a
CIVN766	green (R1)	1.20 ± 0.05 a	7.17 ± 0.44 a	6.77 ± 0.40 c
	white (R2)	0.94 ± 0.05 b	5.50 ± 0.58 b	6.78 ± 0.54 c
	ripe (R3)	0.33 ± 0.03 c	1.56 ± 0.21 c	8.30 ± 0.48 b
	fully ripe (R4)	0.15 ± 0.03 d	1.08 ± 0.15 c	9.30 ± 0.55 ab
	overripe (R5)	0.13 ± 0.01 d	0.75 ± 0.11 c	10.57 ± 0.54 a
Clery	green (R1)	1.18 ± 0.05 a	7.27 ± 0.46 a	6.48 ± 0.27 b
	white (R2)	0.77 ± 0.06 b	3.98 ± 0.44 b	7.07 ± 0.47 b
	ripe (R3)	0.26 ± 0.02 c	1.20 ± 0.18 c	9.04 ± 0.29 a
	fully ripe (R4)	0.21 ± 0.02 c	1.31 ± 0.15 c	10.06 ± 0.36 a
	overripe (R5)	0.19 ± 0.01 c	1.01 ± 0.16 c	9.69 ± 0.34 a
Malwina	green (R1)	1.16 ± 0.05 a	2.67 ± 0.43 a	9.85 ± 0.57 c
	white (R2)	0.80 ± 0.05 b	2.30 ± 0.34 a	11.57 ± 0.38 b
	ripe (R3)	0.58 ± 0.03 c	0.96 ± 0.14 b	12.89 ± 0.56 ab
	fully ripe (R4)	0.56 ± 0.03 c	0.51 ± 0.09 b	12.04 ± 0.31 ab
	overripe (R5)	0.63 ± 0.04 c	0.44 ± 0.07 b	13.31 ± 0.37 a
Variance		F values		
	Cultivar	57.3 ***	28.7 ***	95.5 ***
	Ripening stage	646.1 ***	264.2 ***	47.7 ***
	Cultivar:Ripening stage	10.0 ***	7.2 ***	1.7 *

Different letters indicate statistically significant differences among the ripening stages of each cultivar separately (ANOVA, Duncan's test, $p < 0.05$). ** and ***: significant effect at $p<0.05$and at $p<0.001$, respectively.

cultivars 'Aprica' and 'Malwina', while the malic acid content in the white and green fruit of the cultivars 'Aprica', and in the green fruit in the cultivars 'Asia' and 'Malwina'. The shikimic acid content decreased during ripening in the cultivars 'Aprica', 'CIVN766′ and 'Clery'. However, there were only a few significant differences among the ripening stages of the cultivars 'Malwina' and 'Asia'. The fumaric acid contents showed only a few significant differences among the ripening stages and, compared to the other individual organic acids, showed no significant effect of the ripening stage but a significant effect of the cultivar.

3.4. Sugars

The total sugars content increased during ripening (Table 4), but a decrease at the end of the ripening in overripe fruit was detected in the cultivar 'Clery'. In all studied cultivars, the total sugars content was lower in green fruit (R1) in comparison to the last three ripening stages (R3-R5). The maximum total sugars content (785.76 - 878.82 mg g^{-1}) was detected in the fully ripe fruit (R4) in most of the cultivars except for 'Malwina', where the maximum content (873.49 mg g^{-1}) was detected in the overripe fruit (R5). However, the differences in the content among the last three ripening stages (R3-R5) were not significant in the cultivars 'Aprica', 'Asia' and 'CIVN766′. In the 'Clery' cultivar, the fully ripe fruit (R4) had higher sugar content than the overripe fruit (R5) and in the 'Malwina' cultivar, the ripe fruit (R3) showed lower sugar content than the fully ripe and overripe fruit (R5).

In most cultivars, similar differences were observed in the content of all individual sugars. In the case of glucose, sucrose and fructose, the content was significantly higher in the last three ripening stages (R3-R4) than in the green fruit (R1). In some cultivars, there were also differences among the last three ripening stages and even a decrease in the content was observed. In 'Malwina' and 'Aprica' fruit, the ripe fruit (R3) showed higher content of sucrose than the overripe fruit (R5) and in 'Clery' fruit, the overripe fruit (R5) showed lower glucose content than the fully ripe fruit (R4). Compared to fructose and glucose, the sucrose content showed lower variability among the ripening stages than fructose and glucose. The cultivar did not show a significant effect on the fructose and total sugar content and additionally the interaction of cultivar and ripening stage did not have a significant effect on the total sugar content.

The sugar/acid ratio also increased during ripening, reaching the maximum values either at fully ripe (R4) or the overripe stage (R5). The highest value of sugar/acid ratio (9.62) was detected in the 'CIVN766′ fully ripe fruit (R4), but comparable values were also detected in the overripe stage (R5) and in the 'Clery' fruit at the same ripening stages. The lowest value (5.40) of sugar/acid ratio in fully ripe fruit (R4) was detected in the cultivar 'Malwina'. The cultivar, ripening stage and their interactions showed significant effect on the sugar/acid ratio.

3.5. Pearson correlation

The content of all sugars and organic acids showed a significant correlation with the ripening stage, firmness and ripening index (I_{AD}) except for fumaric acid content (Table 5). Although TSS showed a significant correlation with the total sugars content and the content of individual sugars, the correlation is weaker than the correlation with firmness or I_{AD}. The content of all individual sugars, total sugars content, sugar/acid ratio, TSS and ascorbic acid showed a positive correlation with the ripening stage. On the other hand, total organic acids content, the content of citric, malic and shikimic acids, firmness and ripening index showed a negative correlation with the ripening stage. Also, the ripening index showed a correlation with these parameters, just in the opposite direction than the ripening stage, as there was a strong negative correlation between the ripening index and the ripening stage. Ascorbic acid content showed no or very low correlation with the content of organic acids, but it showed a strong positive correlation with the total sugars content and the content of individual sugars. The individual sugars also showed a strong correlation with each other, with the highest between fructose and glucose content (0.94). Among, the individual organic acids, there were significant correlations, but they were not as strong as in the case of sugars, except for the correlation between citric and malic acid (0.71).

4. Discussion

The ripening index (I_{AD}), as measured using the non-destructive absorbance method, was already tested on different fruit for

Table 3
The content of ascorbic acid, other organic acids and the total organic acid content in 5 ripening stages in 5 strawberry cultivars and the variance of cultivar, ripening stage and their interaction.

Cultivar	Ripening stage	Organic acids content (mg g^{-1} dry weight)					
		Ascorbic	Citric	Malic	Shikimic	Fumaric	Total
Aprica	green (R1)	4.52 ± 0.15 d	144.12 ± 5.63 a	63.32 ± 3.61 a	0.42 ± 0.03 a	0.17 ± 0.01 a	212.55 ± 8.25 a
	white (R2)	5.19 ± 0.15 c	140.08 ± 7.54 a	58.30 ± 4.54 a	0.41 ± 0.02 a	0.15 ± 0.01 b	204.13 ± 10.74 a
	ripe (R3)	5.58 ± 0.18 bc	106.30 ± 5.86 b	29.07 ± 2.21 b	0.30 ± 0.01 b	0.12 ± 0.01 bc	141.37 ± 7.34 b
	fully ripe (R4)	6.42 ± 0.07 a	92.54 ± 4.72 b	26.95 ± 2.03 bc	0.30 ± 0.02 b	0.13 ± 0.01 c	126.34 ± 6.66 b
	overripe (R5)	5.85 ± 0.30 b	76.14 ± 2.53 c	19.59 ± 1.51 c	0.28 ± 0.02 b	0.11 ± 0.01 c	101.97 ± 3.99 c
Asia	green (R1)	2.52 ± 0.21 c	116.03 ± 7.16 a	32.39 ± 1.69 a	0.32 ± 0.02 a	0.14 ± 0.01 c	151.40 ± 8.66 a
	white (R2)	4.02 ± 0.22 b	110.20 ± 7.83 a	29.18 ± 1.07 ab	0.37 ± 0.01 a	0.15 ± 0.01 abc	143.92 ± 8.44 a
	ripe (R3)	4.84 ± 0.21 ab	82.74 ± 6.14 b	27.59 ± 1.00 b	0.32 ± 0.02 a	0.15 ± 0.01 bc	115.64 ± 6.51 b
	fully ripe (R4)	5.97 ± 0.41 a	80.28 ± 3.34 b	24.90 ± 1.27 b	0.35 ± 0.01 a	0.18 ± 0.01 ab	111.68 ± 4.45 b
	overripe (R5)	5.55 ± 0.78 a	77.06 ± 7.48 b	20.27 ± 1.89 c	0.34 ± 0.04 a	0.19 ± 0.02 a	103.41 ± 10.07 b
CIVN766	green (R1)	3.29 ± 0.15 b	98.78 ± 5.74 a	60.74 ± 3.71 a	0.39 ± 0.04 a	0.17 ± 0.01 a	163.37 ± 8.09 a
	white (R2)	4.06 ± 0.27 b	93.91 ± 5.65 a	57.38 ± 3.89 a	0.32 ± 0.01 ab	0.15 ± 0.00 ab	155.82 ± 7.96 a
	ripe (R3)	5.49 ± 0.38 a	72.57 ± 6.55 b	37.38 ± 1.15 b	0.30 ± 0.03 b	0.15 ± 0.01 ab	115.88 ± 7.79 b
	fully ripe (R4)	5.25 ± 0.10 a	65.70 ± 5.51 b	25.29 ± 2.85 c	0.28 ± 0.02 b	0.15 ± 0.01 ab	96.67 ± 8.34 bc
	overripe (R5)	5.32 ± 0.34 a	58.96 ± 4.24 b	25.54 ± 4.08 c	0.26 ± 0.03 b	0.13 ± 0.01 b	90.21 ± 8.20 c
Clery	green (R1)	3.51 ± 0.44 c	100.69 ± 8.92 a	46.37 ± 3.58 a	0.34 ± 0.03 ab	0.09 ± 0.01 a	151.00 ± 12.26 a
	white (R2)	4.00 ± 0.25 bc	106.00 ± 8.70 a	37.58 ± 7.09 a	0.36 ± 0.02 a	0.10 ± 0.01 a	148.05 ± 15.54 a
	ripe (R3)	4.60 ± 0.14 ab	78.28 ± 6.20 b	21.86 ± 1.25 b	0.26 ± 0.01 c	0.10 ± 0.01 a	105.10 ± 6.99 b
	fully ripe (R4)	4.98 ± 0.36 a	71.77 ± 6.95 b	19.56 ± 1.41 b	0.29 ± 0.01 bc	0.10 ± 0.01 a	96.70 ± 7.79 b
	overripe (R5)	5.28 ± 0.09 a	59.98 ± 3.46 b	15.70 ± 0.89 b	0.23 ± 0.02 c	0.10 ± 0.01 a	81.29 ± 4.30 b
Malwina	green (R1)	4.19 ± 0.18 c	133.79 ± 4.57 a	60.36 ± 4.97 a	0.29 ± 0.01 a	0.16 ± 0.01 a	198.79 ± 7.79 a
	white (R2)	4.62 ± 0.18 c	126.23 ± 9.24 ab	49.45 ± 3.98 b	0.23 ± 0.03 ab	0.13 ± 0.02 a	180.66 ± 11.49 ab
	ripe (R3)	4.41 ± 0.13 bc	112.34 ± 8.38 ab	41.04 ± 2.47 b	0.20 ± 0.01 b	0.13 ± 0.01 a	158.12 ± 6.97 bc
	fully ripe (R4)	5.05 ± 0.17 b	105.46 ± 7.29 b	37.42 ± 1.90 bc	0.25 ± 0.03 ab	0.12 ± 0.02 a	148.31 ± 6.74 c
	overripe (R5)	5.75 ± 0.21 a	113.51 ± 3.47 a	47.70 ± 1.62 c	0.24 ± 0.03 ab	0.15 ± 0.02 a	167.35 ± 4.80 bc
Variance		F values					
Cultivar		10.4 ***	37.8 ***	41.7 ***	16.6 ***	24.4 ***	39.8 ***
Ripening stage		42.3 ***	43.9 ***	80.4 ***	13.9 ***	ns	64.9 ***
Cultivar:Ripening stage		2.4 **	1.5 ***	5.8 ***	2.4 **	2.7 ***	2.7 **

Different letters indicate statistically significant differences among the ripening stages of each cultivar separately (ANOVA, Duncan's test, $p < 0.05$). Ns, not significant; ** and ***: significant effect at p<0.01 and at p<0.001, respectively.

monitoring the ripening process (Bonora et al., 2014; Infante et al., 2011; Peifer et al., 2018; Ribera-Fonseca et al., 2016; Smrke et al., 2023; Ziosi et al., 2008), but no study has been performed on strawberries during ripening. In our study, the ripening index decreased during ripening (Table 2), similar to what was previously reported in other fruit (Bonora et al., 2014; Infante et al., 2011; Ziosi et al., 2008) and therefore can serve as a suitable method for distinguishing between the ripening stages in strawberries as well. However, the range depends on the selected cultivar, as seen in the values for the 'Malwina' cultivar. This shows that the method needs to be calibrated for each cultivar. The ripening index results also show a relation to the other parameters (Table 5) - as the ripening index decreases, the ascorbic acid content, sugars content and sugar/acid ratio increase and organic acids content decrease. This measure can therefore be a good guide for distinguishing between the ripening stages. However, the differences between the last three ripening stages are not statistically significant, except for 'CIVN766'..

Additionally, as the fruit ripens, it becomes softer, which is connected with cell wall degradation due to the enzymatic activity of pectin methylesterase and cellulase (Hancock, 2020; Moya-León et al., 2019). As previously reported in other studies (Hwang et al., 2019; Ornelas-Paz et al., 2013), also in our case, the firmness in all cultivars decreased during the ripening process, which can be attributed to increased activity of cellulase and other cell-wall-related enzymes in ripe strawberries (Ramos et al., 2018). However, the enzymatic activity can differ among cultivars, which could explain the differences in firmness among cultivars. As mentioned above, the consequent cell wall degradation products can also serve as precursors for ascorbic acid synthesis in strawberries, which would explain the negative correlation between the firmness and the ascorbic acid content.

Ascorbic acid plays an important role in human nutrition, and among fruit, strawberries show high ascorbic acid content (Giampieri et al., 2014). The average ascorbic acid content is around 50 mg 100 g^{-1} of fresh weight in ripe fruit, but the content also depends on the cultivar (Selamovska, 2014). The ripe fruit of the selected cultivars showed similar content to the previously reported average ascorbic acid content (Table 3), considering an average dry weight content of around 10%. In accordance with previous studies (Cruz-Rus et al., 2011; Fecka et al., 2021; Montero et al., 1996), the ascorbic acid content increased during ripening. The ascorbic acid content positively correlated with the contents of sugars (Table 5), especially with glucose and fructose, which is in agreement with a previous study on strawberries (Pistón et al., 2017), which suggests these sugars are important precursors of ascorbic acid in plants. The content also increased as the fruit softened, as the ascorbic acid in strawberries can also be produced from cell wall polymers via L-galacturonic acid (Cruz-Rus et al., 2011; Mellidou et al., 2019). The ascorbic acid content does not differ much among the last three ripening stages. However, the few differences detected can be significant for choosing the right ripening stage for processing, as higher ascorbic acid content can mean lower color stability, which is a concern in processed strawberry products (Gössinger et al., 2014). However, these small differences among the last three ripening stages were not consistent among the cultivars. Therefore, each cultivar needs to be assessed in order to identify the optimal ripening stage for processing.

Both sugars and organic acids contribute to a great extent to the taste perception of the fruit (Kader, 1991). Among sugars, it is important to study also the content of the individual sugars as each has a different level of sweetness (Fotirić Akšić et al., 2019). However, in plants, sugars also serve as the major metabolic constituents for carbon skeleton construction, as energy supply, but they are also involved in the signaling

Table 4
The sugar content and sugar/acid ratio in 5 ripening stages in 5 strawberry cultivars and the correlation between sugar content and the variance of cultivar, ripening stage and their interaction.

Cultivar	Ripening stage	Sugars content (mg g^{-1} dry weight)				Sugar/acid ratio
		Sucrose	Glucose	Fructose	Total	
Aprica	green (R1)	79.43 ± 10.47 c	177.02 ± 11.52 b	191.58 ± 10.09 d	448.04 ± 30.72 c	2.18 ± 0.20 d
	white (R2)	125.73 ± 7.29 ab	296.44 ± 32.96 a	270.53 ± 14.79 c	692.70 ± 47.25 b	3.58 ± 0.40 c
	ripe (R3)	138.58 ± 11.63 a	291.79 ± 24.00 a	309.17 ± 22.26 bc	739.55 ± 57.43 ab	5.44 ± 0.28 b
	fully ripe (R4)	146.74 ± 9.64 a	330.61 ± 19.02 a	370.60 ± 27.85 a	847.95 ± 55.54 a	7.17 ± 0.61 a
	overripe (R5)	107.03 ± 3.10 b	297.30 ± 5.41 a	330.67 ± 4.41 ab	735.00 ± 10.12 ab	7.72 ± 0.39 a
Asia	green (R1)	74.96 ± 13.85 c	172.76 ± 7.83 d	198.13 ± 8.16 c	445.85 ± 29.29 c	3.13 ± 0.16 c
	white (R2)	103.80 ± 8.90 bc	229.46 ± 3.85 c	268.41 ± 6.03 b	601.67 ± 11.40 b	4.16 ± 0.24 c
	ripe (R3)	125.71 ± 11.67 ab	285.33 ± 13.17 b	307.96 ± 15.75 ab	719.00 ± 40.09 ab	6.22 ± 0.72 b
	fully ripe (R4)	157.31 ± 15.65 a	347.42 ± 19.51 a	374.09 ± 20.13 a	878.82 ± 54.89 a	7.81 ± 0.96 ab
	overripe (R5)	155.26 ± 21.43 a	309.94 ± 30.00 ab	355.84 ± 41.04 a	821.04 ± 91.05 a	8.26 ± 0.27 a
CIVN766	green (R1)	88.12 ± 12.88 b	195.69 ± 2.58 d	185.59 ± 4.65 b	469.39 ± 16.70 b	2.96 ± 0.15 c
	white (R2)	98.41 ± 20.45 b	225.99 ± 14.08 cd	232.91 ± 19.42 b	557.30 ± 53.08 b	3.65 ± 0.24 c
	ripe (R3)	156.31 ± 12.73 a	262.63 ± 19.83 bc	306.58 ± 15.49 a	725.53 ± 39.09 a	6.73 ± 0.61 b
	fully ripe (R4)	180.18 ± 8.41 a	317.92 ± 15.72 a	346.73 ± 21.79 a	844.83 ± 40.46 a	9.62 ± 0.97 a
	overripe (R5)	182.37 ± 18.46 a	290.09 ± 17.29 ab	299.09 ± 19.86 a	771.55 ± 49.03 a	9.29 ± 0.59 a
Clery	green (R1)	66.34 ± 3.78 c	165.14 ± 15.02 d	181.41 ± 16.89 c	412.89 ± 35.41 d	2.82 ± 0.15 b
	white (R2)	113.83 ± 8.10 ab	245.85 ± 11.74 c	263.09 ± 13.16 b	622.77 ± 32.66 c	4.47 ± 0.28 b
	ripe (R3)	102.05 ± 9.23 b	295.53 ± 13.84 ab	316.53 ± 8.61 a	714.11 ± 28.07 ab	7.39 ± 0.85 a
	fully ripe (R4)	136.78 ± 11.05 a	310.48 ± 15.36 a	338.50 ± 16.54 a	785.76 ± 28.68 a	8.95 ± 0.97 a
	overripe (R5)	112.15 ± 12.06 ab	269.50 ± 6.32 bc	301.51 ± 7.20 a	683.16 ± 21.75 bc	9.09 ± 0.42 a
Malwina	green (R1)	103.21 ± 5.99 c	193.40 ± 14.88 c	185.96 ± 15.48 d	482.56 ± 33.42 c	2.48 ± 0.14 d
	white (R2)	130.87 ± 5.42 abc	246.58 ± 9.90 b	249.93 ± 8.57 c	627.38 ± 20.88 b	3.72 ± 0.25 c
	ripe (R3)	142.12 ± 12.00 a	272.30 ± 9.54 b	272.53 ± 8.27 c	686.95 ± 17.67 b	4.54 ± 0.20 b
	fully ripe (R4)	136.11 ± 11.88 ab	332.30 ± 7.56 a	346.94 ± 7.60 b	815.35 ± 24.97 a	5.40 ± 0.34 a
	overripe (R5)	110.32 ± 7.80 bc	365.28 ± 14.44 a	397.89 ± 12.22 a	873.49 ± 31.78 a	5.20 ± 0.08 a
Variance		F values				
Cultivar		5.5 *	2.5 *	ns	ns	17.8 ***
Ripening stage		24.3 ***	64.8 ***	80.3 ***	70.3 ***	106.1 ***
Cultivar:Ripening stage		2.7 *	2.0 *	1.7 *	ns	2.4 **

Different letters indicate statistically significant differences among the ripening stages of each cultivar separately (ANOVA, Duncan's test, $p < 0.05$). Ns, not significant; *, ** and ***: significant effect at $p<0.05$, $p<0.01$ and $p<0.001$, respectively.

during fruit ripening (Durán-Soria et al., 2020; Jia et al., 2013). In agreement with previous studies (Hwang et al., 2019; Moing et al., 2001; Montero et al., 1996; Sturm et al., 2003; Topcu et al., 2022), the sugars content increased during ripening of strawberries (Table 4). Montero et al. (1996) also reported a decreasing trend in the content of sugars towards the end of the ripening, which was also observed in our study in the cultivar 'Clery'. This decrease did not seem to affect the ascorbic content in the fruit suggesting that the ascorbic acid synthesis from cell wall constituents plays a significant role towards the end of the ripening in some cultivars as the fruit continues to soften at these ripening stages. In all the selected cultivars, glucose and fructose are the predominant sugars, and they make up approximately 80% of the total sugar content, which is similar to results obtained by Kafkas et al. (2007). However, in some cultivars, sucrose can be the predominant sugar (Lee et al., 2018). Therefore, it is necessary to assess each cultivar individually as it maybe affects not only the sugar metabolism but also other metabolic pathways where sugars serve as precursors. The content of individual sugars is mainly influenced by the activity of invertase, which regulates the balance between sucrose and hexoses (Hancock, 2020; Topcu et al., 2022). However, the changes in the individual sugar contents can differ among cultivars as can be seen in the increase in sucrose content at the end of ripening in 'Aprica' and 'Malwina', which suggests that there are differences in the expression of genes, which are involved in the sugar metabolism as it has been reported before by Topcu et al. (2022). TSS is a parameter used to evaluate the maturity of the fruit at harvest (Azam et al., 2019). In our case, fruit at the last three ripening stages of all cultivars showed higher TSS than 7%, which is recommended level for acceptable flavor (Mitcham et al., 1996), and in some cultivars ('Clery' and 'Malwina'), this level was already reached in the white ripening stage. Although the TSS results correlate with the total sugars content detected by HPLC, the correlation coefficient was low, showing that other soluble compounds may affect the TSS value, and the TSS value does not accurately measure the three main sugars contributing to the strawberry flavor.

Also, organic acids content changed during ripening and the content decreased during ripening (Table 3), but the individual organic acids can show a different trend during ripening. Our results are in agreement with other studies (Hwang et al., 2019; Ornelas-Paz et al., 2013; Sturm et al., 2003; Vandendriessche et al., 2013), which also reported a decline in the organic acids content. However, this can be very dependent on the cultivar, as other studies detected an increase in organic acids content for specific cultivars during ripening (Kafkas et al., 2007; Sturm et al.,

Table 5
Pearson correlation among the ripening stage, ripening index, firmness, TSS and primary metabolite contents.

	Ripening stage[a]	Ripening index (I_{AD})	Firmness	TSS	Ascorbic acid	Citric acid	Malic acid	Shikimic acid	Fumaric acid	Total organic acid content	Sucrose	Glucose	Fructose	Total sugars content
Ripening stage[a]	na													
Ripening index (I_{AD})	−0.84 ***	na												
Firmness	−0.83 ***	0.81 ***	na											
TSS	0.52 ***	−0.29 ***	−0.66 ***	na										
Ascorbic acid	0.65 ***	−0.65 ***	−0.60 ***	0.26 **	na									
Citric acid	−0.58 ***	0.61 ***	0.46 ***	ns	−0.18 *	na								
Malic acid	−0.64 ***	0.76 ***	0.62 ***	ns	−0.30 ***	0.71 ***	na							
Shikimic acid	−0.39 ***	0.31 ***	0.53 ***	−0.63 ***	ns	0.40 ***	0.35 ***	na						
Fumaric acid	ns	ns	ns	−0.23 **	ns	0.30 ***	0.28 ***	0.54 ***	na					
Total organic acid content	−0.64 ***	0.70 ***	0.55 ***	ns	−0.21 **	0.96 ***	0.87 ***	0.41 ***	0.32 ***	na				
Sucrose	0.49 ***	−0.57 ***	−0.53 ***	0.23 **	0.58 ***	ns	−0.25 **	ns	0.21 *	−0.19 *	na			
Glucose	0.71 ***	−0.68 ***	−0.67 ***	0.41 ***	0.70 ***	−0.26 **	−0.37 ***	ns	ns	−0.31 ***	0.66 ***	na		
Fructose	0.76 ***	−0.72 ***	−0.70 ***	0.36 ***	0.74 ***	−0.31 ***	−0.47 ***	ns	ns	−0.38 ***	0.64 ***	0.94 ***	na	
Total sugars content	0.73 ***	−0.72 ***	−0.71 ***	0.38 ***	0.74 ***	−0.28 ***	−0.42 ***	ns	ns	−0.33 ***	0.78 ***	0.97 ***	0.97 ***	na
Sugar/acid	0.78 ***	−0.82 ***	−0.69 ***	0.26 **	0.51 ***	−0.79 ***	−0.76 ***	−0.34 ***	−0.17 *	−0.83 ***	0.53 ***	0.64 ***	0.69 ***	0.69 ***

[a] after assigning numerical values from 1 to 5 to the ripening stages R1 to R5, respectively; ns, not significant, ** and ***: significant correlation at $p<0.01$ and at $p<0.001$, respectively.

2003; Zhang et al., 2011). Based on our results, the total organic acids content and the content of the individual organic acids show high variability based on the cultivar, which suggests that the organic acid synthesis is highly specific to each cultivar.

The decrease in organic acids and an increase sugars content during ripening resulted in a high sugar/acid ratio in ripe strawberries, as has been previously reported (Hwang et al., 2019; Sturm et al., 2003) and is in agreement with our results (Table 4) The sugar/acid ratio that provides the best flavor in strawberries is in the range of 8 to 11 (MacNaeidhe, 2001). In our study, the highest sugar/acid ratio in ripe fruit was detected in the fruit of the cultivar 'CIVN766', followed by the cultivar 'Clery' and the fruit of these cultivars would fit within the ideal range, but only starting with the fully ripe fruit. This shows that it is necessary to distinguish between the early stage of ripe and the fully ripe stage in order to harvest the fruit that would provide the best sugar/acid ratio and, consequently, the best taste.

5. Conclusion

Our study showed that the content of organic acids and sugars changes significantly during ripening. The ascorbic acid content, sugars content and sugar/acid ratio increased, and the organic acids content, firmness and ripening index decreased as the fruit ripened. Most of the changes showed variability based on the cultivar, except for the fructose content and the total sugars content. These differences can have an impact on the fruit quality for fresh consumption or processing, which shows that it is important to distinguish between the early ripe, fully ripe and overripe stages. The ripening index proved as a good rapid non-destructive method for distinguishing between the unripe (green and white) and ripe fruit and correlates well with the content of sugars, ascorbic acid and other organic acids except for fumaric acid. However, the ripening index results showed that the method needs to be adjusted for each cultivar as they can show different values for the same ripening stage. For most cultivars, the optimal ripening index of ripe fruit is lower than 0.33, but for the cultivar 'Malwina', the value can be between 0.56 – 0.63. Additionally, firmness also showed as a good indicator of the ripening stage and content of sugars and organic acids, and the value of firmness of ripe strawberry fruit for the studied cultivars should be below 1.70 N. On the other hand, TSS showed weaker correlations with the content of sugars and cannot serve as an indicator of the total content of organic acids. Although these indicators can serve to distinguish unripe fruit from ripe fruit, there is still a need for further analysis or indicators to distinguish between the last three ripening stages as there can be significant differences in the sugars and organic acids content.

Funding

This work was supported by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 956257; and the Slovenian Research and Innovation Agency (ARIS) within the research programme Horticulture (P4-0013).

CRediT authorship contribution statement

Kristyna Simkova: Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. **Robert Veberic:** Writing – review & editing. **Metka Hudina:** Funding acquisition, Resources, Writing – review & editing. **Mariana Cecilia Grohar:** Investigation. **Massimiliano Pelacci:** Investigation. **Tina Smrke:** Investigation. **Tea Ivancic:** Investigation. **Nika Cvelbar Weber:** Conceptualization, Investigation, Resources. **Jerneja Jakopic:** Conceptualization, Methodology, Supervision, Validation, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2024.112843.

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