Adv. Hort. Sci., 2024 38(1): 47-62 DOI: 10.36253/ahsc-15033

AHS
Advances in Horticultural Science

A biostimulant complex comprising molasses, *Aloe vera* extract, and fish-hydrolysate enhances yield, aroma, and functional food value of strawberry fruit

K. Wise [1, 2], J. Selby-Pham [1, 2 (*)], T. Simovich [3, 4], H. Gill [1]

[1] *School of Science, RMIT University, Bundoora, VIC 3083, Australia.*
[2] *Nutrifield, Sunshine West, Victoria, VIC 3020, Australia.*
[3] *School of Engineering, RMIT University, Melbourne, VIC 3000, Australia.*
[4] *PerkinElmer Inc., Glen Waverley, VIC 3150, Australia.*

Key words: Antioxidant, *Fragaria*, hydroponics, phenolics, super food.

(*) **Corresponding author:**
jamie@nutrifield.com

Citation:
WISE K., SELBY-PHAM J., SIMOVICH T., GILL H. 2024 - *A biostimulant complex comprising molasses, Aloe vera extract, and fish-hydrolysate enhances yield, aroma, and functional food value of strawberry fruit.* - Adv. Hort. Sci., 38(1): 47-62.

Data Availability Statement:
All relevant data are within the paper and its Supporting Information files.

Competing Interests:
The authors declare no competing interests.

Received for publication 9 August 2023
Accepted for publication 21 September 2023

Abbreviations: A = achromatic; ATC = automatic temperature compensation; B = blue; BC = Biostimulant complex; C = cyan; EC = electrical conductivity; F-C = Folin-Ciocalteu; FTIR = Fourier transformed infrared; G = green; GAE = gallic acid equivalent; GLM = general linear model; GM = genetic modification; M-IR = mid-infrared; O = orange; PCA = principle component analysis; Pi = pink; PLS-DA = partial least squares-discriminant analysis; Pu = purple; QE = quercetin equivalent; R = red; RWC = relative water content; sPLS-DA = sparse partial least squares-discriminant analysis; SSC = soluble solids content; TP = total phenolics; UATR = universal attenuated total reflectance; W = white; Y = yellow.

Abstract: Strawberry is a popular functional food due to the presence of antioxidant and anti-inflammatory phytochemicals. Enhancing this functional food value is an opportunity to improve consumer health, but strategies to do so cannot compromise yield or organoleptic properties, which are highest priorities for farmers and consumer, respectively. One promising strategy is the supplementation of fertiliser regimens with biostimulants, which are non-nutritive substances associated with species-specific improvements to crop growth, yield, and quality. Accordingly, the impacts of a biostimulant complex (BC) containing molasses, *Aloe vera* extract, and fish-hydrolysate is characterised herein for its potential to impact strawberry growth, yield, quality, and functional food value. Results indicated that BC treatment significantly increased ($p < 0.05$) plant biomass and canopy area (growth), total fruit count and weight per plant (yield), fruit aroma and colour (quality), and antioxidant potential (functional food value). The results presented highlight the potential utility of biostimulants to the strawberry sphere, providing a strategy to enhance the fruit to the benefit of both farmers and consumers.

1. Introduction

The genus *Fragaria* is comprised of 25 species of small flowering plants known as strawberries, which are widely cultivated for their edible fruits (Hirakawa *et al.*, 2014). Of these 25 species, the hybrid octoploid *Fragaria* x *ananassa*, is the most popular variety, accounting for 60% of the worlds strawberry fruit production (Amil-Ruiz *et al.*, 2011). The global strawberry industry is a profitable and growing industry, with world production outputs in 2020 equal to 40.76 million tonnes (FAO, 2022). Whilst strawberry fruits are primarily consumed as a fresh fruit, they are also popular additions to processed foods such jams, juices, dairy products, and flavoured drinks, making strawberries one of the most popular and versatile global agricultural products (Moraga *et al.*, 2006; Basu *et al.*, 2014).

The drivers of consumer perception of strawberry fruit quality are their physical features, organoleptic properties, nutritional value, and added secondary health benefits (functional food properties) such as antioxidant, anti-inflammatory, and anti-hypertensive activities (Basu *et al.*, 2014). Improving these measures is therefore desirable to consumers who would experience higher quality produce and potentially added health benefits from increased functional food properties. The antioxidant activity of strawberry fruits is a particular driver of the fruit's popularity, as this functional food property is associated with a range of benefits including improvements to cardiovascular health (Giampieri *et al.*, 2015), neurodegeneration (Esposito *et al.*, 2002), cancer (Zhang *et al.*, 2008), and type 2 diabetes (da Silva Pinto *et al.*, 2010). The antioxidant activity of the fruit is attributed to the high concentrations of polyphenols including flavonoids, anthocyanins, and ellagitannins, and vitamins such as ascorbic and folic acid, which have been shown to vary in concentration in fruits depending on cultivar, storage, processing, and cultivation system (Basu *et al.*, 2014; Afrin *et al.*, 2016). In addition to improving the functional properties of the fruit, strawberry yield and organoleptic improvements are also significant opportunities to benefit the strawberry industry.

Processing (Moraga *et al.*, 2006), plant breeding (Diamanti *et al.*, 2012), and cultivation practises (Akhatou *et al.*, 2014) have all been shown to impact fruit sensory properties such as firmness and taste, however, breeding is slow and costly, and processing is unsuitable to the fresh fruit sector. Therefore, modifications to farming practises could be potentially fast and versatile interventions to strawberry cultivation, to improve fruit quality metrics - functional food potential and organoleptic desirability - and thereby enhance customer perception of quality and value returned to farmers. Accordingly, implementation of biostimulants continues to generate interest as a fast and environmentally friendly improvement to cultivation practises, as these inputs are non-nutritive compounds or substances which beneficially impact plant growth, development, or yield (Du Jardin, 2015). Various biostimulants have been associated with improvements to fruit quality in a range of species, however effects are plant-species and dosage dependent, requiring further exploration of these effects in target crops such as strawberry (Rodrigues *et al.*, 2020; Wise *et al.*, 2020).

Molasses is a by-product from the sugar industry, which is rich in simple and complex carbohydrates, proteins, amino acids, organic acids, and Maillard reaction by-products (melanoidins) (Najafpour and Shan, 2003; Chandra *et al.*, 2008). It has been used as an additive in agriculture and has demonstrated biostimulant effects including increasing yield in beetroot (Nadeeka and Seran, 2020), sugar cane (Srivastava *et al.*, 2012) and spinach (Pyakurel *et al.*, 2019), improved biomass growth in maize (Shahzad *et al.*, 2018) and sorghum (Suliasih and Widawati, 2016), enhanced salinity tolerance in thyme (Koźmińska *et al.*, 2021), and improvements to disease resistance in various species (Welbaum *et al.*, 2004). Similarly, molasses distillery effluent, which is more dilute but similarly comprised to molasses, also has demonstrated biostimulant benefits such as increased yield in banana (Thakare *et al.*, 2013) and sweet pepper (Gaafar *et al.*, 2019), increased nutrient uptake in radish (Hatano *et al.*, 2016), improved growth and development of rapeseed (Li *et al.*, 2020), altered antioxidant activity in cabbage (Bimova and Pokluda, 2009) and black bean (Elayaraj, 2014), as well as increased heavy metal uptake by common reed and sedge for potential applications to bioremediation (Nagy *et al.*, 2020). Accordingly, utilisation of molasses as a biostimulant input for strawberry farming may be expected to positively impact a range of yield, growth, and quality measures.

Aloe vera extracts are also complex mixtures containing plant nutrients, vitamins, enzymes, amino acids, sugars, hormones, and hormone-like compounds (Ishartati *et al.*, 2019; Cortés *et al.*, 2021). The biostimulant effects associated with application

of *Aloe vera* extracts are varied, including increased propagation efficiency and growth of Populus tree clones (El Sherif, 2017), eucalyptus tissue cultures (Hendi, 2021), and grape vine cuttings (Uddin *et al.*, 2020), improved growth, biomass, and oil content of sweet basil (Hamouda *et al.*, 2012), increased growth, yield, oil content, and nutritional content of caraway (Khater *et al.*, 2020), improved growth and chlorophyl content of fenugreek (Al-Yasiri *et al.*, 2021), increased leaf growth and terpene content in lavender (El Sherif *et al.*, 2020), enhanced fruit yield and nutritional content of okra (Hemalatha *et al.*, 2018 a, b), and dose-dependent positive and negative effects on cereal germination (Baličević *et al.*, 2018). Accordingly, *Aloe vera* extracts are utilised as fertiliser additives to improve plant growth and have the potential to improve a range of attributes when added to strawberry plants during cultivation.

Agricultural amino acids can be purified or complex mixtures, which are often extracted (hydrolysed) from animal, plant, or microbial products (Calvo *et al.*, 2014). Legume-derived hydrolysates are common sources of amino acids and have a range of beneficial effects associated with their use during cultivation including increased vegetative growth, yield, and secondary metabolite production in capsicum (Ertani *et al.*, 2014), and increased yield (Colla *et al.*, 2017), nutritional content (Colla *et al.*, 2017; Rouphael *et al.*, 2017), firmness (organoleptic property) (Mirabella *et al.*, 2021), and antioxidant activity (Caruso *et al.*, 2019) in tomato. Furthermore, amino acids derived from fish-hydrolysate have demonstrated increased yield in tomato (García-Santiago *et al.*, 2021), pig blood-hydrolysate increased phenolic and antioxidant properties of lettuce (Zhou *et al.*, 2022), and a commercial amino acid product was shown to increase antioxidant activity in the leaves of *Aloe vera* (Ardebili *et al.*, 2012). Amino acids are also associated with improved nutrient uptake in plants, either directly through provision of organic-N or indirectly through stimulation of soil microbes or chelation of nutrients (Callahan *et al.*, 2007; Halpern *et al.*, 2015). Accordingly, addition of amino acids during strawberry cultivation may demonstrate biostimulant effects to improve a range of measures and thereby benefit consumers and/ or farmers.

Noting the potential benefits of molasses, *Aloe vera* extract, and fish-hydrolysate as biostimulants during strawberry cultivation, the aim of this study was to explore impacts to strawberry growth, yield, and quality associated with the supply of a complex of these biostimulants. This involved the hydroponic growth of strawberry plants with application of the biostimulant complex, followed by temporal assessment to impacts to fruit yield, in addition to end point fruit quality measures such as fruit antioxidant potential, and fruit sensory profile. Characterisation of the effect of these biostimulants to strawberry has the potential to benefit both strawberry farmers and consumers by providing a cost efficient and easily implemented farming strategy to enhance the value of strawberry yields.

2. Materials and Methods

Plant materials and growth conditions

Stock tubes of strawberry plants (*Fragaria* x *ananassa* 'Albion'), supplied by Sunny Ridge Strawberry Farm Pty Ltd. (Boneo, VIC, Australia), were planted into 15 cm pots in Coco perlite substrate (Nutrifield Pty Ltd., Melbourne, VIC, Australia) and maintained in indoor growth rooms for 5 weeks with Coco A&B nutrients (Nutrifield Pty Ltd., Melbourne, VIC, Australia) at pH = 5.8, and EC = 1.0. Fertigation was delivered via a flood and drain system, wherein trays containing potted plants were filled with fertigation liquid to 75% the height of the pots and subsequently drained. Plants were grown under 315W ceramic metal-halide horticultural lamps (315W CMH Pro 4200K, Indoor Sun, Melbourne, VIC, Australia), with a Recom 315W Ballast (Lucius, Melbourne, VIC, Australia), and light:dark (L:D) photoperiod of 12 hours day and 12 hours night. Environmental conditions were restricted to day temperature and relative humidity of 21.5°C, and 70%, respectively, and night temperature and relative humidity of 18°C, and 51%, respectively. After 5 weeks, plants were separated into treatment groups and re-potted into 30 cm square pots with Coco perlite substrate and a top layer (2 cm) of Hydro Clay (Nutrifield Pty Ltd., Melbourne, VIC, Australia).

Treatment and fertigation programme

Twelve plants were split into 2 groups (n = 6): control group, receiving Coco A&B nutrients as per usage instructions, and treatment group, receiving Coco A&B nutrients as per usage instructions plus the biostimulant complex (BC) comprising molasses (10% w v^{-1}), *Aloe vera* extract (2.5% v v^{-1}), and fish-hydrolysate (5% v v^{-1}) at 2 mL L^{-1} during weeks 10-18. Fertigation was delivered to plants via a recirculating drip-irriga-

tion system (4 × 4 L h^{-1} $dripper^{-1}$ $plant^{-1}$) as described in Table 1. The elemental composition, phytohormone profile, and metabolite profile of the biostimulant complex is provided in supplemental tables S1, S2, and S3, respectively.

Table 1 - Fertigation programme for strawberry plants during treatment (weeks 6-18)

Weeks	Fertigation programme (split evenly throughout the day)
6-11	3 × 10 min
12-13	4 × 10 min
14-18	6 × 10 min

Vegetative measurements

Leaf colour (*L*a*b**) was measured using a CR-400 Chroma Meter colourimeter (Konica Minolta, Tokyo, Japan). Canopy area was measured using the smartphone application Easy Leaf Area Free (Easlon *et al.*, 2014). At the conclusion of the harvest period leaf and crown number were counted, and final fresh- and dry-weight measurements were taken for leaf and non-leaf tissues. Vegetative tissues were dried in an ED 53 oven (BINDER, Tuttlingen, Germany) at 70°C for 3 days.

Harvesting and fruit measures

Ripe fruit - defined as BBCH = 87 according to Wise *et al.* (2022) - were harvested, immediately measured (weight and length), and placed into a DT5600 Food Dehydrator (Sunbeam, FL, USA) at 55°C for 7 days. Strawberry fruit length at harvest was measured as the perpendicular distance from the centre of the calyx to the tip of the receptacle. Fruit width at harvest was measured by image analysis using ImageJ (Schneider *et al.*, 2012). In short, image global pixel scale was set based on known fruit length and the 'measure' feature within the ROI manager was utilised to measure the widest fruit diameter.

Determination of pH and Brix of crude fruit extract

A crude extract was prepared by pressing fresh strawberry fruits through four layers of muslin cloth and subsequently passing the filtrate through an additional single layer of muslin cloth. An automatic temperature compensation (ATC) portable refractometer (Sugar/Brix Refractometer 0-32% 300001, Super Scientific Ltd., Scottsdale, AZ, USA) was used to measure Brix (°Bx) of the pure crude extract. A 1/1000 dilution of the crude extract in water was used to measure pH (Sension+ MM 374 GLP 2 channel Laboratory Meter with Sension + 5014T pH liquid combination electrode with silver ion barrier, HACH, Loveland, CO, USA).

Fruit phytochemical extraction

Whole fruit extraction was carried out with adaptations to the method described in Chandra *et al.* (2014). In short, the dried strawberry fruits were pulverised in a 'multigrinder II' (Sunbeam, FL, USA) and then extracted in 8 mL ethanol (100%) per g pulverised fruit. The extraction was carried out in a sonicator at 40°C for 10 min and then filtered through 7-10 µm membrane filter paper with 0.1% ash content (Westlab, Ballarat, VIC, Australia). The ethanol filtrate was evaporated in a 100°C water bath to achieve a dried extract, which was then resolubilised in 5 mL 5% methanol in a sonicator at 40°C for 10 min.

Determination of total phenolic and flavonoid content

Total phenolic (TP) and flavonoid content was determined as per the Folin-Ciocalteu (F-C) method, and the aluminium chloride colorimetric method, respectively, which were adapted from those described in Chandra *et al.* (2014). In short, TP content was determined by combining 100 µL of resolubilised extract with 100 µL F-C reagent, 300 µL 8% w v^{-1} saturated sodium carbonate solution, and 1.5 mL distilled water. Solutions were reacted under light in a PS-10A sonicator (Jeken, Dongguan, China) at 40°C for 30 min. The absorbance was measured at 765 nm and phenolic content was calculated as gallic acid equivalent per gram dry fruit (GAE g g^{-1} d.w.). Total flavonoid content was determined by combining 1 mL of the resolubilised extract with 1 mL 10% (w v^{-1}) aluminium chloride. The solutions were reacted at room temperature for 1 h, and then absorbance was measured at 420 nm. The results were calculated as quercetin equivalent per gram dry fruit (QE mg g^{-1} d.w.). All absorbance measurements were analysed using the DR 5000™ UV-Vis Spectrophotometer (HACH, Loveland, CO, USA).

Sensory perception testing

A blinded test was conducted to explore if the biostimulant treatment was associated with changes to sensory perception of fruits. Participants (n = 6) were asked to score the fruits (on a 9-point scale)

based on their texture, taste, and aroma (Table 2).

Plant image colour analysis

Colour data of strawberry fruit images was extracted as per Wise *et al.* (2022), wherein individual pixels were categorised as either achromatic (A, light grey-black), blue (B), cyan (C), green (G), orange (O), pink (Pi), purple (Pu), red (R), white (W), or yellow (Y), based on maximal similarity to predefined colours.

Mid-infrared (M-IR) analysis

Infrared spectra of the dried strawberry fruits were collected using a Spectrum 2 FTIR spectrophotometer (PerkinElmer Inc., Waltham, MA, USA) equipped with a Universal Attenuated Total Reflectance (UATR) accessory with diamond crystal. Spectra were collected with a 4 cm^{-1} resolution over the 500-7000 cm^{-1} range with four accumulations to produce an averaged spectrum. For data analysis the 4000-7000 cm^{-1} range was excluded. Spectral data were standardised to 1875 cm^{-1} prior to analysis, corresponding with the region of the sample spectra which has minimal influence from the presence of water as indicated by the water absorption spectra (NIST, 2022), presented in the NIST Chemistry WebBook (Linstrom and Mallard, 2001).

Statistical analysis

Analyses implemented during exploration of treatment effects on individual measures included general linear model (GLM), Tukey's test 95% confidence grouping analyses, Anderson-Darling normality test, and Mood's median test in the Minitab 19 statistical software package (Minitab Inc., State College, PA, USA). Analyses implemented to explore treatment effects on the profiles of plant measures (and fruit M-IR spectra) were heatmap analysis, dendrogram, principle component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), sparse PLS-DA (sPLS-DA), and fold change (1.3-fold threshold), using the web-tool Metaboanalyst 5.0 (Chong *et al.*, 2019). The profiles of plant measures were normalised within Metaboanalyst using the cube root transformation and pareto scaling functions, while M-IR data was transformed by auto-scaling. Mean changes in sensory perception was assessed by a repeated-measures t-test, performed in Minitab 19. Replicates per analysis are presented in supplemental table S1.

3. Results

Profile analyses (vegetative, yield, and quality)

A range of growth, yield, and quality measures were taken per plant (27 measures total) to assess the impact of the BC treatment on strawberry plants (Table S4). Principal component analysis (Fig. 1A) and PLS-DA (Fig. 1B), identified clearly distinct 95% confidence regions in the trait profiles between BC and control treated plants, which is consistent with the clustering of sample profiles by treatment within the dendrogram (Fig. 1C) and heatmap (Fig. S1). Heatmap analysis (Fig. S1 and Table S5) identified two clusters within the 27 plant measures (13 vegetative, 7 yield, and 8 quality), one cluster with minimal difference between treatments (6 vegetative, 5

Table 2 - Sensory perception scoring matrix

	Sensory perception								
	Poor				Acceptable				Optimal
Aroma - Desirability	1	2	3	4	5	6	7	8	9
Taste - Desirability	1	2	3	4	5	6	7	8	9
	Low				Moderate				High
Mouthfeel - Firmness	1	2	3	4	5	6	7	8	9
Mouthfeel - Juiciness	1	2	3	4	5	6	7	8	9
	None				Moderate				Strong
Aroma - Intensity	1	2	3	4	5	6	7	8	9
Taste - Sweet	1	2	3	4	5	6	7	8	9
Taste - Sour/acid	1	2	3	4	5	6	7	8	9
Taste - Intensity	1	2	3	4	5	6	7	8	9

yield, and 4 quality), and one cluster of measures with large difference between treatments (7 vegetative, 2 yield, and 4 quality). The cluster associated with large differences was comprised of the measures: leaf count (number), leaf fresh weight (g f.w.), leaf dry weight (g d.w.), above ground (non-leaf) fresh weight (g f.w.), above ground (non-leaf) dry weight (g d.w.), canopy area (cm^2), and leaf colour-a (V2, V3, V4, V6, V7, V9, and V11, respectively), total fruit harvested (g) and fruit harvested (number) (Y5 and Y6, respectively), and fruit GAE per fruit dry weight ($g \cdot g^{-1}$ d.w.), Brix %, fruit pH, and fruit water % (Q1, Q2, Q3, and Q5, respectively).

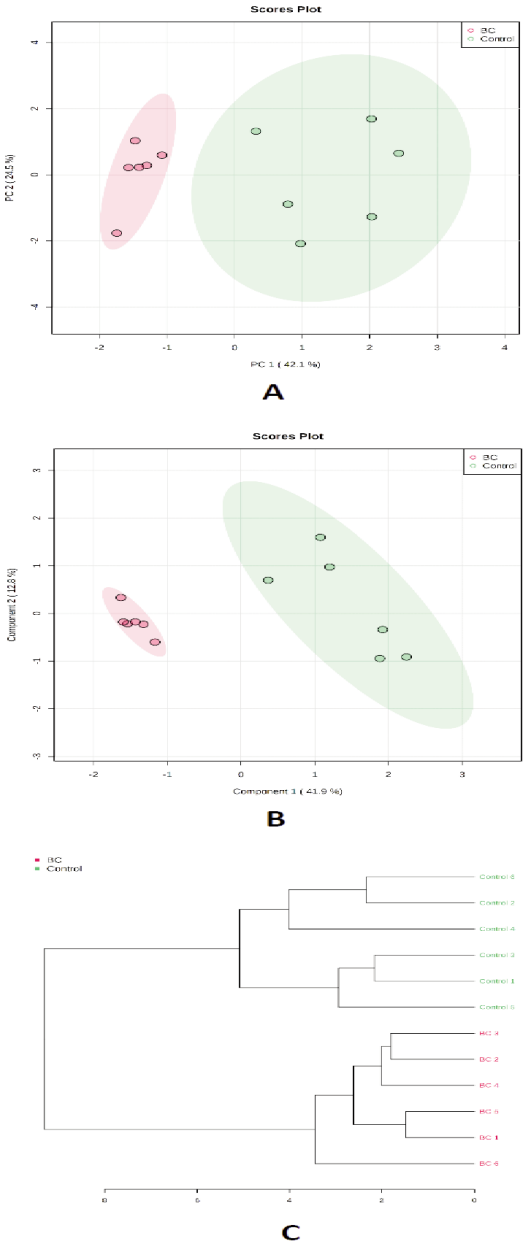

Fig. 1 - Effect of biostimulant complex (BC) on strawberry (*Fragaria x ananassa* 'Albion') trait profiles. (A) Principal component analysis (PCA), and (B) partial least squares-discriminant analysis (PLS-DA), with shading indicating 95% confidence regions. C) Dendrogram indicating hierarchical clustering (Ward clustering algorithm) based on Euclidean distance of plants based on measured traits. Trait values measured from 6 biological replicates.

Vegetative analyses

Treatment with BC had significant impacts on several vegetative measures (Table S4). The greatest effect was seen for leaf measures including: leaf number ($p < 0.001$) which increased by 107.4% (over two-fold) from 15.67 in control to 32.5 in treatment (Fig. 2A), leaf dry weight ($p = 0.002$) which increased by 66.7% from 7.4 g d.w. in control to 12.4 g d.w. in treatment (Fig. 2B), and canopy area ($p = 0.001$) which increased by 51.8% from 401.4 cm^2 in control to 609.3 cm^2 for treatment (Fig. 2C). Whilst vegetative measures tended to increase, no significant change ($p = 0.205$) was identified for leaf water content (Table S4). Furthermore, the BC treatment increased ($p = 0.001$) non-leaf aerial dry weight by 64% from 5.7 g d.w. for control to 9.4 g d.w. for treatment (Table S4), and with marginal significance ($p = 0.057$) increased crown number by 35% from 3.3 to 4.5 (Fig. 2D), whilst not significantly affecting leaf

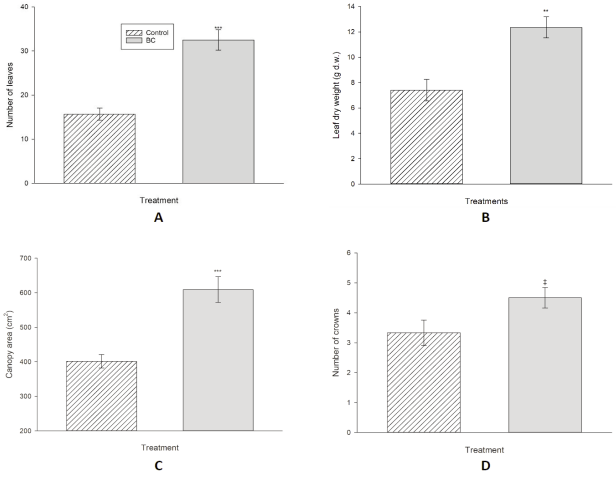

Fig. 2 - Effect of biostimulant complex (BC) treatment on vegetative growth measures of strawberry (*Fragaria x ananassa* 'Albion'). A) Number of leaves, (B) leaf dry weight, (C) canopy area, and (D) number of crowns compared between control and BC treated plants. Data presented mean ± standard deviation of 6 biological replicates. Significant differences are indicated by ‡ for $p < 0.1$; * for $p < 0.05$; ** for $p < 0.01$; *** for $p \leq 0.001$ calculated by Student's t-test.

colour measures *L**, *a**, and *b** (Table S4).

Yield analyses

The biostimulant complex significantly increased the yields of strawberry plants in terms of total weight of fruits per plant (p = 0.038), and number of fruits per plant (p = 0.035), whilst not significantly (p = 0.666) affecting individual fruit weight (Table S4). The average weight of total fruits harvested per plant increased by 50.7% from 128.3 g for control to 193.3 g for treatment (Fig. 3A), while the average number of fruits harvested per plant increased by 56.9% from 12.0 for control to 18.8 for treatment (Fig. 3B). Whilst no changes were observed for individual fruit weight, changes to fruit shape were observed wherein BC treatment significantly (p = 0.013) increased fruit length (Table S4) from 30.33 mm to 35.48 mm (17% increase), whilst fruit width was unchanged (p = 0.446). Harvest timing was normally distributed for control plants (p = 0.268) but not for treatment plants (p = 0.038), however no significant difference between treatments was identified in median fruit harvest timing (p = 0.971, Fig. 3C).

Fruit quality analyses

Chemical analysis of strawberry fruits identified that BC treatment significantly increased fruit water content (p = 0.001) wherein fruit water content from treatment plants was 90.2%, whilst fruits from control plants had 89.06% water content (Fig. 4A). Additionally, the BC treatment resulted in significant changes to fruit image colour profiles, wherein R% increased from 29.66% for control to 39.22% (p = 0.001), G% increased from 0.38% for control to 1.01% (p = 0.001), and O% decreased from 28.90% for control to 20.79% (p = 0.006), whilst Y% (p = 0.371) and A% (p = 0.186) were not significantly impacted (Fig. 4B). The fruit colour measures B, Pi, C, W, and Pu accounted for on average less than 0.01% of pixels within images and so were not explored during analyses. No significant difference was observed for Brix content (p = 0.941) between treatment or control plants (Fig. 4C). The biostimulant complex treatment resulted in a marginally (defined as $0.1 < p < 0.05$) significant (p = 0.055) increase to pH of diluted crude fruit extract, from 4.2 to 4.4 (Table S4).

It was identified that the BC treatment had significant affects (p = 0.029) on TP content (Fig. 4D) with a 32% increase from 0.0139 GAE $g \cdot g^{-1}$ d.w. for control to 0.0184 GAE g g^{-1} d.w. for treatment, whilst not significantly (p = 0.534) affecting flavonoid content (Fig. 4E). Fruit quality perception was analysed by a blinded sensory perception test on a 9-point scale, which identified that the BC treatment significantly (p = 0.043) increased fruit mouthfeel firmness ('firmness'), from 4.2 for control to 5.5 for treatment, whilst not significantly impacting mouthfeel juiciness ('juiciness'), or fruit taste measures (Table S6).

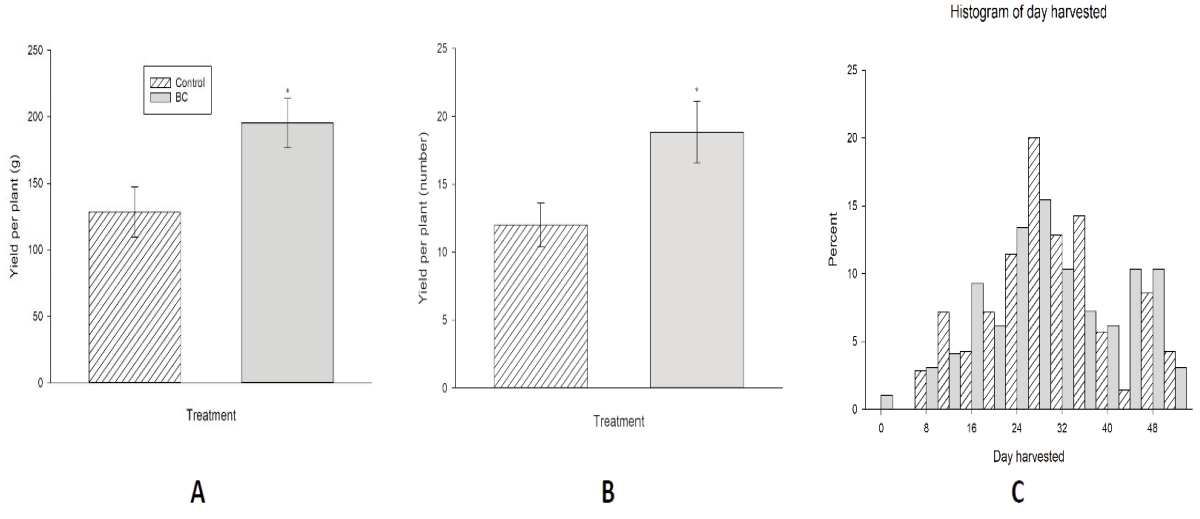

Fig. 3 - Effect of biostimulant complex (BC) treatment on yield and harvest timing of strawberry fruits (*Fragaria x ananassa* 'Albion'). Fruit yield per plant by (A) weight, and (B) count compared between control and BC treated plants. A-B) Data presented as mean ± standard deviation of 6 biological replicates. Significant differences are indicated by * for $p < 0.05$ calculated by Student's t-test. C) Histogram of harvest timing from 72 fruits from control plants and 97 fruits from BC treated plant.

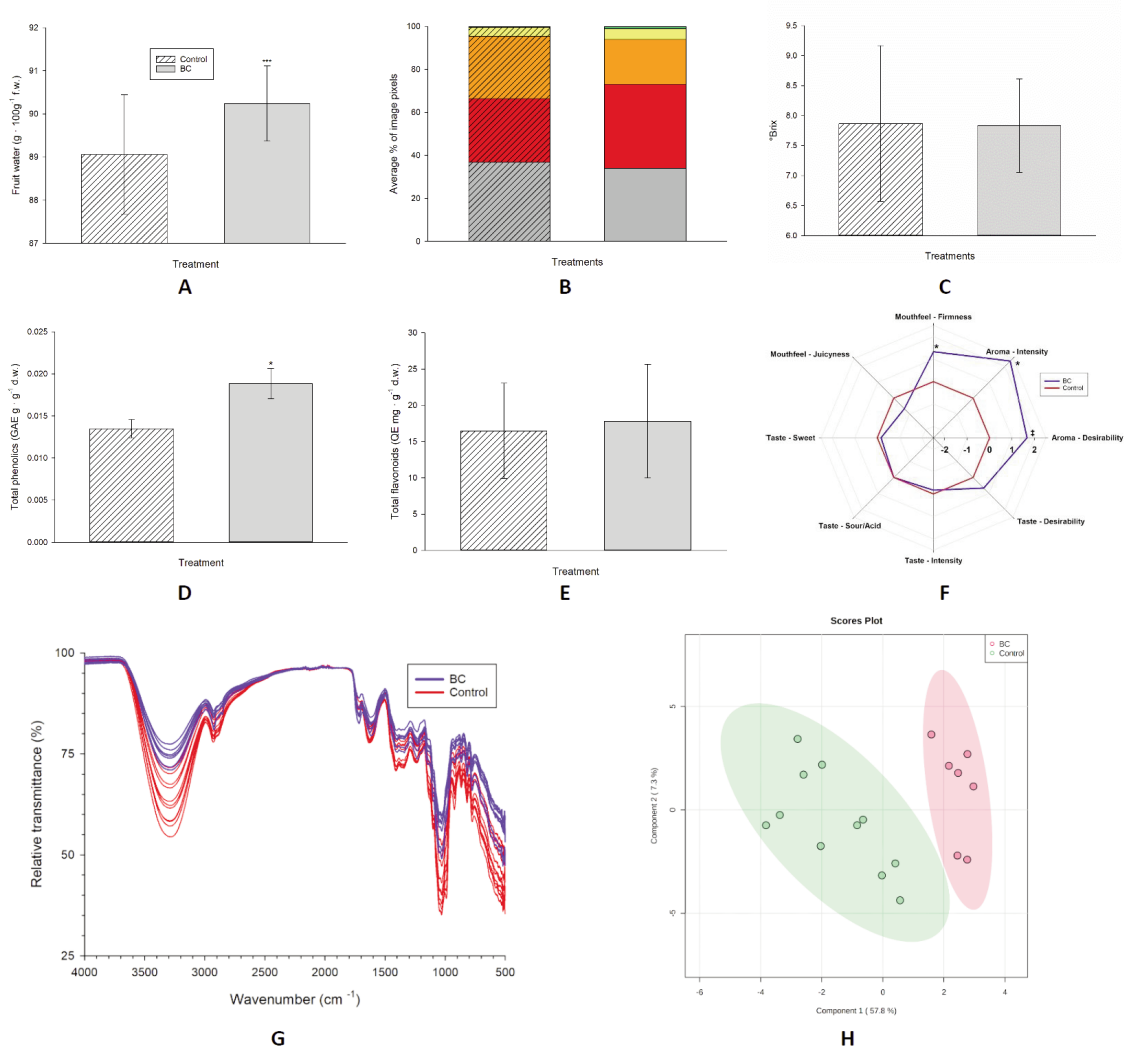

Fig. 4 - Effect of biostimulant complex (BC) treatment on quality and sensory perception of strawberry (*Fragaria* x *ananassa* 'Albion') fruits. A) Fruit water (22 and 27 biological replicates for control and BC, respectively), (B) colour profile (22 and 27 biological replicates for control and BC, respectively with each colour represented as their respective colour), (C) Brix (6 and 13 biological replicates for control and BC, respectively), (D) total phenolics (22 and 27 biological replicates for control and BC, respectively), and (E) total flavonoids (22 and 27 biological replicates for control and BC, respectively) compared between control and BC treated plants. A, C-E) Data represented as mean ± standard deviation. F) Comparison of mean scores (6 participants) for blind sensory perceptions of fruit from control and BC treated plants. A-E) Solid bars represent control and dashed bars represent BC treatment. A, C-F) Significant differences are indicated by ‡ for $p < 0.1$; * for $p < 0.05$; ** for $p < 0.01$; *** for $p \leq 0.001$ calculated by Student's t-test (A-E), and repeated-measures t-test (F). G) Mid-infrared (M-IR) spectra of dehydrated strawberry fruits analysed between 500-4000 cm^{-1} (7 and 11 biological replicates for control and BC, respectively). H) Sparse partial least squares-discriminant analysis (sPLS-DA) of M-IR spectra with shading indicating 95% confidence regions.

Furthermore, the BC treatment significantly (p = 0.040) increased fruit aroma intensity from 3.5 to 5.2, and had a marginally significant (p = 0.067) effect to aroma desirability which increased from 5.0 to 6.7 (Fig. 4F).

Mid-infrared spectrometry analysis between 500-4000 cm^{-1} identified biostimulant induced changes to fruit chemical composition (Fig. 4G). Principle component analysis (PCA: Fig. S2) and PLS-DA (Fig. S3) did not identify significant differences between the profile of spectral bins between treatment groups, while sPLS-DA (Fig. 4H) indicated significant differences in a subset of the wavelength's measured. Comparison of the average spectra of each treatment (Fig. S4), identified a region between 1024-1048 cm^{-1} with the highest fold change (>1.3) between treatments (Fig. S5).

4. Discussion and Conclusions

Crop productivity is an important factor in food production when considering the growing global population and uncertainties associated with climate change (Lobell and Gourdji, 2012). Accordingly, new strategies to increase crop outputs are highly sought after, with a focus on fast acting benefits which do not contribute to environmental degradation. Whilst genetic modification (GM) continues to benefit many crop sectors, the costs, time, and resources required to develop approved GM food crops is a significant hurdle. Accordingly, biostimulants are becoming increasingly popular additives during plant growth due to their benefits to crop productivity, natural origin, cost, and ease of use (Parađiković *et al.*, 2019). Herein a naturally derived biostimulant complex comprising molasses, *Aloe vera* extract, and fish-hydrolysate exemplifies these beneficial effects by increasing the growth and yield of strawberry.

Application of the BC was shown to increase vegetative biomass and canopy area measures (Fig. 2A-C), potentially associated with the provision of zeatin (cytokinin) - the only phytohormone detected in both the BC concentrate and its associated reservoir solution (Table S2) - which has been shown to increase shoot and root growth when applied exogenously to strawberry (Debnath, 2006). Additionally, the BC treatment resulted in a marginally significant (p = 0.057) increase to crown number (Fig. 2D). The crown is the central node of the strawberry plant from which roots, leaves, inflorescence, and additional crowns form (Savini *et al.*, 2005; Poling, 2012). As crowns are the base of future inflorescence formation, their number correlates strongly with fruit yield (Strik and Proctor, 1988; Kadir *et al.*, 2006) and is therefore an important factor for strawberry cultivation. Additionally, application of the BC was observed to increase both yield-weight (Fig. 3A, p = 0.038) and yield-number (Fig. 3B, p = 0.035) per plant - potentially associated with the increased crown number - which are crucial measures of profitability for farmers. Accordingly, the increases in yield outputs reported herein (Fig. 3A and 3B) support the utilisation of these biostimulants by the strawberry industry and thereby presents as a low-cost, effective, and easily integrated farming strategy to improve growth and yield.

Furthermore, changes to strawberry shape - increased length (Fig. S6A, p = 0.013) but not weight (Fig. S6B, p = 0.666) or width (Fig. S6C, p = 0.446) - was observed from application of the BC. Changes to fruit size and shape can be impactful to farmer sales due to the compliance standards imposed by supermarkets. For strawberry fruits, compliance is generally determined according to diameter (USDA, 2006; Woolworths Supermarkets Ltd, 2010), which is also the highest correlating size measurement (R^2 = 0.93) with consumer preference, however, length is the second highest correlation (R^2 = 0.77) (Lewers *et al.*, 2020), suggesting that longer fruits of unchanged width may be considered preferable by consumers. Accordingly, application of this biostimulant complex to strawberries during growth can benefit farmers by improving yield and improve customer perceptions of quality through altered fruit size.

Whilst the aforementioned changes to fruit size are likely to be impactful to consumer perception of fruit quality, organoleptic properties and colour features are also highly correlative with strawberry quality perception (Lewers *et al.*, 2020). Organoleptic measures include taste, texture, mouthfeel, and aroma, which are conferred to the fruit through its chemical composition (Saliba-Colombani *et al.*, 2001). Common measures associated with taste include soluble solids content (SSC), titratable acidity (Wozniak *et al.*, 1996), and pH (Gunness *et al.*, 2009). Herein no significant change was detected for brix (p = 0.941), a measure of SSC (Saranwong *et al.*, 2003), which is consistent with the results from the sensory assessment wherein no significant change (p = 0.822) was observed in the correlated measure, sweetness (Jouquand *et al.*, 2008). Similarly, sour perception may have been expected to change with pH (Jouquand *et al.*, 2008), and whilst a marginally significant change was observed for fruit pH (p = 0.055), no significant change was reported from panellists for the sensory measure sour (p = 1.000). This may be explained by the apparently small change of 0.11 pH of diluted extract, which is consistent with the findings of Harker *et al.* (2002) wherein a minimum shift of 0.14 pH of apple extract was required for participants to perceive a change in apple acidity. These results suggest that utilisation of the BC during strawberry cultivation may increase yield without compromising quality. Noting that a growing point of consumer dissatisfaction is the reduction in food flavour and aroma due to the prioritisation of more profitable crop attributes such as yield and visual aesthetic (Klee, 2010; Tieman *et al.*, 2017), these results support the utilisation of biostimulants as being advantageous to both farmers and consumers.

Fruit mouthfeel and texture are associated with cell wall composition (Caner *et al.*, 2008) and thickness (Szczesniak and Smith, 1969), water content (Cordenunsi *et al.*, 2002), and pH (Plotto *et al.*, 2010). Additionally, Salentijn *et al.* (2003) and Wang *et al.* (2021) have shown that increased expression of genes associated with lignin production is associated with increased firmness of strawberry fruit. Accordingly, the significant increase in mouthfeel-firmness (p = 0.043) may relate to the presence of caffeic acid in the BC (Table S3), which may internally translocate via the phloem and xylem (Zhang and Hamauzu, 2004; Ishimaru *et al.*, 2011) and has been shown to increase lignin production in soybean (Bubna *et al.*, 2011). Richter (1978) identified that plant cell turgidity is highly sensitive to changes in relative water content (RWC), with flaccidity (loss of turgidity) to full turgor occurring over the narrow range of 5% RWC. Accordingly, as fruit firmness is impacted by turgidity (Szczesniak and Smith, 1969; Raharjo *et al.*, 1998), the 1% increase in fruit water content (p = 0.001) identified from the BC treatment may explain the observed increase in the sensory measure for firmness. Whilst this apparently minor change in fruit water content (Fig. 4A) may have impacted perceived firmness (Fig. 4F), it is however not surprising that this small change in fruit water volume (150 µL, based on 1% of 10.5 g average fruit fresh weight) was below sensory perception thresholds to impact perceived juiciness, as juiciness is generally considered as the amount of liquid released during chewing (Roger Harker *et al.*, 2003; Harker *et al.*, 2006).

Aroma - also referred to as odour - is the detection and recognition of compounds within the olfactory system and is conferred by the presence of volatile compounds (El Hadi *et al.*, 2013). In strawberry, aroma is predominantly attributed to esters, furanones, terpenes, and sulfur compounds (Yan *et al.*, 2018). As with fruit flavour, aroma is often seen by consumers as a sacrifice for higher yields (Klee, 2010; Tieman *et al.*, 2017), which necessitates the need for methods to improve aroma, or improve yields without compromising this measure. Herein a significant difference (p = 0.040) was observed for aroma intensity and a marginally significant (p = 0.067) difference was observed for aroma desirability (Fig. 4F), suggesting that the BC treatment may have altered the volatile contents or profiles of the fruits. The M-IR analysis presented in figure 4G revealed a narrow region between 1024–1048 cm^{-1} with a high fold change, which has been associated with chemicals in the classes of phenolic alkyl-aryl ethers, aryl phenolic ester tannins (Abbas *et al.*, 2017), pyranose rings (saccharides), alkyl amines, and alcohols (Lingegowda *et al.*, 2012). Furthermore, of these classes of compounds, esters are one of the most abundant volatiles in strawberries (Yan *et al.*, 2018) and have been shown to correlate strongly with strawberry fruit liking (Fan *et al.*, 2021). Whilst the scoring of odour desirability alone herein was only marginally significant, this association with overall fruit liking combined with the other changes reported herein, is likely to contribute to an overall improvement to fruit quality perception from the BC treatment. Accordingly, BC treatment may have resulted in changes to ester levels to enhance the sensory aroma properties of strawberry fruits, which is also reflected by M-IR profile changes over a narrow region. These outcomes address consumer concerns for losses in aroma associated with prioritisation of more profitable traits, by demonstrating that BC treatment increases both yield and aroma.

Finally, fruit appearance, which includes colour (Crisosto *et al.*, 2003) and damage (Jaeger *et al.*, 2018), is a major impactor to consumer perception of quality and purchasing decision, as it is the first impression of a fruit. Biostimulant complex treatment resulted in significant increases to the colour measures for red (p = 0.001) and green (p = 0.001) and reductions in orange (p = 0.006). Strawberry colour is conferred by the presence (amount and types) of anthocyanins, which have a strong pH-colour relationship (Holcroft and Kader, 1999). The predominant anthocyanins in strawberry are pelargonidins and cyanidins (Andersen *et al.*, 2004) which appear red at low pH and with increasing pH change to colourless, yellow, or blue forms which affects the overall appearance of the fruit (Holcroft and Kader, 1999). Whilst a marginally significant (p = 0.055) increase of 0.11 pH in diluted fruit extract was associated with the BC treatment, this degree of change is small relative to the change observed in Wang *et al.* (2015) wherein pH shifts of 1.0 were associated with noteworthy changes to colour. Accordingly, pH is likely not the driver of the observed changes in fruit colour, which may instead be attributed to changes in the concentrations or ratios of anthocyanins present (Yoshida *et al.*, 2002). Nevertheless, strawberry colour is a driver of consumer preference, as evidence by Wang *et al.* (2017) wherein an 'ideal red' colour was the preference for

fresh strawberry fruit, and by Wendin *et al.* (2019) which showed that red colour intensity had a significant positive impact to consumer preference for woodland strawberries. These studies suggest that the increased red from BC treatment reported herein (Fig. 4B) for common garden strawberries may also be associated with increased consumer preference.

Unlike organoleptic measures and colour features which are directly detectible by consumers and therefore impactful to quality perception and preference, other properties such as nutritional and functional food value should also be considered as targets for improvement during production and cultivation as their increased presence may benefit consumer health (Selby-Pham *et al.*, 2017; Topolska *et al.*, 2021). Due to the presence of many beneficial polyphenols and vitamins, strawberries are considered to be a functional food which can reduce hypertension, postprandial oxidative stress, inflammation, and hyperglycaemia when consumed (Giampieri *et al.*, 2015). Furthermore, agricultural practises such as fertiliser form (Tomic *et al.*, 2016), cultivation system (D'evoli *et al.*, 2010), and beneficial microbes (Rahman *et al.*, 2019) have been shown to impact phytochemical profiles and antioxidant activities in strawberries. Accordingly, the results presented herein are similar to these observations, wherein altered cultivation conditions through application of the BC was shown to impact phytochemical concentrations which are associated with functional activity when consumed.

Herein two methods were utilised to measure functional compounds in strawberry, and whilst the aluminium chloride method has relatively good specificity for flavonoid quantification (Mabry *et al.*, 1970), the F-C method is a non-specific method, which quantifies total reducing capacities (antioxidant activity) rather than specific classes of compounds (Magalhães *et al.*, 2008). Accordingly, the 32% increase (p = 0.029) in TP and unchanged (p = 0.534) flavonoid contents (Fig. 4D and 4E, respectively) reported herein indicates that the biostimulant treatment increased the antioxidant activity of the fruits in the non-flavonoid portion of the phytochemical profile. Aaby *et al.* (2007) identified that the largest contributors to strawberry antioxidant activity were ascorbic acid, and the polyphenolics ellagitannins, and anthocyanins, which accounted for 24%, 19%, and 13% of strawberry antioxidant capacities, respectively. Anthocyanins are also the class of compounds conferring the majority of strawberry colour (Yoshida *et al.*, 2002), which also changed in response to the biostimulant application (Fig. 4B), discussed above. As noted, changes in anthocyanin concentrations or ratios may explain the changes in colour observed for biostimulant treated fruits, and changes to anthocyanins may also affect antioxidant activities of the fruit extracts (Cerezo *et al.*, 2010). The distinguishing feature of anthocyanins, is their multiple aromatic rings with hydroxyl groups (phenolic) structure, derived from the flavylium ion (Khoo *et al.*, 2017). Whilst the carbon bonds of these aromatic rings (C=C) and the hydroxyl groups (OH) are associated with IR absorption at 1654 cm^{-1} and 677 cm^{-1}, and 3385 cm^{-1}, respectively, the C-O bond connecting the hydroxyl to the aromatic ring is associated with wavenumber 1029 cm^{-1} (Wahyuningsih *et al.*, 2017), which is contained within the range of wavenumbers (1024-1048 cm^{-1}) identified herein as having increased from the BC treatment (Fig. 4H and S5). Accordingly, it appears that the BC treatment induced changes in the strawberry anthocyanin content or profile, which would be consistent with the changes observed for TP (Fig. 4D), colour (Fig. 4B), and M-IR spectra (Fig. 4G). Furthermore, the changes in TP (antioxidant activity) correspond with improved functional food potential of these fruits (Giampieri *et al.*, 2015), which may impart greater health benefits than control strawberries when consumed. Implementation of this complex is therefore a promising improvement to strawberry cultivation practises and may be an additional tool available to farmers to improve yields and quality of produce for consumers.

Biostimulants are an exciting development in agriculture which have the potential to improve crop yields and quality, whilst not requiring significant time and money to substantially alter crop outputs, by contrast to alternative strategies such as genetic modification and selective breeding. However, species-specific efficacies of popular biostimulants even when applied to commonly grown food crops are often not well understood. Accordingly, this project characterised the impacts of a complex containing the biostimulants molasses, *Aloe vera* extract, and fish-hydrolysate when applied to strawberry in a hydroponic, environmental-controlled growth system. The results demonstrated that application of the complex increased crop yield, vegetive growth, and fruit quality measures including aroma and functional food value. Accordingly, utilisation of biostimulants within farming practises

has demonstrated potential to increase crop outputs for farmers whilst enhancing the quality of foods for consumers.

Acknowledgements

The authors would like to thank: Sunny Ridge Strawberry Farm for generously supplying the strawberry plants, Todor Vasiljevic for lending the Minolta colourimeter for leaf colour assessment, and Abigail Jabines, Munira, Louis Fenech, Darcy Simondson-Tammer, and Joshua Masci for their assistance in method development and data collection.

References

AABY K., EKEBERG D., SKREDE G., 2007 - *Characterization of phenolic compounds in strawberry (*Fragaria × ananassa*) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity*. - J. Agric. Food Chem., 55: 4395-4406.

ABBAS O., COMPÈRE G., LARONDELLE Y., POMPEU D., ROGEZ H., BAETEN V., 2017 - *Phenolic compound explorer: a mid-infrared spectroscopy database*. - Vib. Spectrosc., 92: 111-118.

AFRIN S., GASPARRINI M., FORBES-HERNANDEZ T.Y., REBOREDO-RODRIGUEZ P., MEZZETTI B., VARELA-LOPEZ A., GIAMPIERI F., BATTINO M., 2016 - *Promising health benefits of the strawberry: A focus on clinical studies*. - J. Agric. Food Chem., 64: 4435-4449.

AKHATOU I., FERNÁNDEZ RECAMALES Á., 2014 - *Influence of cultivar and culture system on nutritional and organoleptic quality of strawberry*. - J. Sci. Food Agric 94, 866-875.

AL-YASIRI Z.A.N., AL-ALWANI B., AL-JANABI J.K.A., 2021 - *Detection of genetic polymorphisms using random amplified polymorphic DNA (RAPD)-PCR in fenugreek (*Trigonella foenum-graecum*) plants after seed treatment with biotic and abiotic agents*. - J. Pure Appl. Microbiol., 15 1409-1421.

AMIL-RUIZ F., BLANCO-PORTALES R., MUNOZ-BLANCO J., CABALLERO J.L., 2011 - *The strawberry plant defense mechanism: a molecular review*. - Plant Cell Physiol., 52: 1873-1903.

ANDERSEN Ø.M., FOSSEN T., TORSKANGERPOLL K., FOSSEN A., HAUGE U., 2004 - *Anthocyanin from strawberry (*Fragaria ananassa*) with the novel aglycone, 5-carboxypyranopelargonidin*. - Phytochemistry, 65: 405-410.

ARDEBILI Z.O., MOGHADAM A.R.L., ARDEBILI N.O., PASHAIE A.R., 2012 - *The induced physiological changes by foliar application of amino acids in* Aloe vera *L. plants*. - Plant Omics, 5: 279.

BALIČEVIĆ R., RAVLIĆ M., LUCIĆ K., TATAREVIĆ M., LUCIĆ P., MARKOVIĆ M., 2018 - *Allelopathic effect of* Aloe vera *(L.) Burm. F. on seed germination and seedlings growth of cereals, industrial crops and vegetables*. - Poljoprivreda, 24: 13-19.

BASU A., NGUYEN A., BETTS N.M., LYONS T.J., 2014 - *Strawberry as a functional food: an evidence-based review*. - Crit. Rev. Food Sci. Nutr., 54: 790-806.

BIMOVA P., POKLUDA R., 2009 - *Impact of organic fertilizers on total antioxidant capacity in head cabbage*. - Hortic. Sci., 36: 21-25.

BUBNA G.A., LIMA R.B., ZANARDO D.Y.L., DOS SANTOS W.D., FERRARESE M.D.L.L., FERRARESE-FILHO O., 2011 - *Exogenous caffeic acid inhibits the growth and enhances the lignification of the roots of soybean (*Glycine max*)*. - J. Plant Physiol., 168: 1627-1633.

CALLAHAN D.L., KOLEV S.D., RICHARD A., SALT D.E., BAKER A.J., 2007 - *Relationships of nicotianamine and other amino acids with nickel, zinc and iron in* Thlaspi *hyperaccumulators*. - New Phytol., 176: 836-848.

CALVO P., NELSON L., KLOEPPER J.W., 2014 - *Agricultural uses of plant biostimulants*. - Plant and Soil, 383: 3-41.

CANER C., ADAY, M.S., DEMIR, M., 2008 - *Extending the quality of fresh strawberries by equilibrium modified atmosphere packaging*. - Eur. Food Res. Technol., 227: 1575-1583.

CARUSO G., DE PASCALE S., COZZOLINO E., CUCINIELLO A., CENVINZO V., BONINI P., COLLA G., ROUPHAEL Y., 2019 - *Yield and nutritional quality of Vesuvian Piennolo tomato PDO as affected by farming system and biostimulant application*. - Agron., 9: 505.

CEREZO A.B., CUEVAS E., WINTERHALTER P., GARCIA-PARRILLA M., TRONCOSO A., 2010 - *Isolation, identification, and antioxidant activity of anthocyanin compounds in Camarosa strawberry*. - Food Chem., 123: 574-582.

CHANDRA R., BHARAGAVA R.N., RAI V., 2008 - *Melanoidins as major colourant in sugarcane molasses based distillery effluent and its degradation*. - Bioresour. Technol., 99: 4648-4660.

CHANDRA S., KHAN S., AVULA B., LATA H., YANG M.H., ELSOHLY M.A., KHAN I.A., 2014 - *Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study*. - Evid. Based Complementary Altern. Med., 2014: 253875.

CHONG J., WISHART D.S., XIA J., 2019 - *Using MetaboAnalyst 4.0 for comprehensive and integrative metabolomics data analysis*. - Curr. Protoc. Bioinformatics, 68: e86.

COLLA G., CARDARELLI M., BONINI P., ROUPHAEL Y., 2017 - *Foliar applications of protein hydrolysate, plant and seaweed extracts increase yield but differentially modulate fruit quality of greenhouse tomato*. - HortScience,

52: 1214-1220.

CORDENUNSI B.R., OLIVEIRA DO NASCIMENTO J.R., GENOVESE M.I., LAJOLO F.M., 2002 - *Influence of cultivar on quality parameters and chemical composition of strawberry fruits grown in Brazil*. - J. Agric. Food Chem., 50: 2581-2586.

CORTÉS J.S.A., GODOY J.A., RICA C., MORA R.M.S., 2021 - *Metabolites with phytohormonal potential in vegetal crops: biotechnologycal aplications*. - Environ. Technol., 2: 18-47.

CRISOSTO C.H., CRISOSTO G.M., METHENEY P., 2003 - *Consumer acceptance of 'Brooks' and 'Bing' cherries is mainly dependent on fruit SSC and visual skin color*. - Postharvest Biol. Technol., 28: 159-167.

DA SILVA PINTO M., DE CARVALHO, J.E., LAJOLO F.M., GENOVESE M.I., SHETTY K., 2010 - *Evaluation of antiproliferative, anti-type 2 diabetes, and antihypertension potentials of ellagitannins from strawberries (*Fragaria × ananassa *Duch.) using* in vitro *models*. - J. Med. Food, 13: 1027-1035.

DEBNATH C.S., 2006 - *Zeatin overcomes thidiazuron-induced inhibition of shoot elongation and promotes rooting in strawberry culture* in vitro. - J. Hortic. Sci. Biotechnol., 81: 349-354.

D'EVOLI L., TAROZZI A., HRELIA P., LUCARINI M., COCCHIOLA M., GABRIELLI P., FRANCO F., MORRONI F., CANTELLI-FORTI G., LOMBARDI-BOCCIA G., 2010 - *Influence of cultivation system on bioactive molecules synthesis in strawberries: spin-off on antioxidant and antiproliferative activity*. - J. Food Sci., 75: C94-C99.

DIAMANTI J., CAPOCASA F., BALDUCCI F., BATTINO M., HANCOCK J., MEZZETTI B., 2012 - *Increasing strawberry fruit sensorial and nutritional quality using wild and cultivated germplasm*. - PlosOne,7: e46470.

DU JARDIN P., 2015 - *Plant biostimulants: Definition, concept, main categories and regulation*. - Sci. Horticult., 196: 3-14.

EASLON H.M., BLOOM A.J., 2014 - *Easy leaf area: Automated digital image analysis for rapid and accurate measurement of leaf area*. - Appl. Plant Sci., 2: 1400033.

EL HADI M.A.M., ZHANG F.-J., WU F.-F., ZHOU C.-H., TAO J., 2013 - *Advances in fruit aroma volatile research*. - Molecules, 18: 8200-8229.

EL SHERIF F., ALBOTNOOR N., YAP Y.-K., MELIGY A., KHATTAB S., 2020 - *Enhanced bioactive compounds composition in* Lavandula officinalis *in-vitro plantlets using NaCl and* Moringa oleifera*,* Aloe vera *and* Spirulina platensis *extracts*. - Ind. Crops Prod., 157: 112890.

EL SHERIF F., 2017 - Aloe vera *leaf extract as a potential growth enhancer for* Populus *trees grown under in vitro conditions*. - Am. J. Plant Biol., 2: 101-105.

ELAYARAJ B., 2014 - *Impact of sugar factory effluent on germination, biochemical and antioxidant enzyme activity of Black gram L. Hepper variety Vamban 3*. - Int. J. Int. J. Cur. Tr. Re., 3: 84-89.

ERTANI A., PIZZEGHELLO D., FRANCIOSO O., SAMBO P., SANCHEZ-CORTES S., NARDI S., 2014 - Capsicum chinensis *L. growth and nutraceutical properties are enhanced by biostimulants in a long-term period: chemical and metabolomic approaches*. - Front. Plant Sci., 5: 375.

ESPOSITO E., ROTILIO D., DI MATTEO V., DI GIULIO C., CACCHIO M., ALGERI S., 2002 - *A review of specific dietary antioxidants and the effects on biochemical mechanisms related to neurodegenerative processes*. - Neurobiol. Aging, 23: 719-735.

FAN Z., HASING T., JOHNSON T.S., GARNER D.M., SCHWIETERMAN, M.L., BARBEY, C.R., COLQUHOUN T.A., SIMS C.A., RESENDE M.F., WHITAKER V.M., 2021 - *Strawberry sweetness and consumer preference are enhanced by specific volatile compounds*. - Hortic. Res., 8: 66.

FAO, 2022 - *FAOSTAT: Strawberries*. UNdata. - FAO, https://data.un.org/

GAAFAR M.S., EL-SHIMI N.M., HELMY M., 2019 - *Effect of foliar and soil application of some residuals of sugar cane products (molasses and vinasses) with mineral fertilizer levels on growth, yield and quality of sweet pepper*. - Menoufia J. Plant Prod. ,4: 353-373.

GARCÍA-SANTIAGO J.C., LOZANO CAVAZOS C.J., GONZÁLEZ-FUENTES J.A., ZERMEÑO-GONZÁLEZ A., RASCÓN ALVARADO E., ROJAS DUARTE A., PRECIADO-RANGEL P., TROYO-DIÉGUEZ E., PEÑA RAMOS, F.M., VALDEZ-AGUILAR, L.A., 2021 - *Effects of fish-derived protein hydrolysate, animal-based organic fertilisers and irrigation method on the growth and quality of grape tomatoes*. - Biol. Agric. Hortic., 37: 107-124.

GIAMPIERI F., FORBES-HERNANDEZ T.Y., GASPARRINI M., ALVAREZ-SUAREZ J.M., AFRIN S., BOMPADRE S., QUILES J.L., MEZZETTI B., BATTINO M., 2015 - *Strawberry as a health promoter: an evidence based review*. - Food Funct., 6: 1386-1398.

GUNNESS P., KRAVCHUK O., NOTTINGHAM S.M., D'ARCY B.R., GIDLEY M.J., 2009 - *Sensory analysis of individual strawberry fruit and comparison with instrumental analysis*. - Postharvest Biol. Technol., 52: 164-172.

HALPERN M., BAR-TAL A., OFEK M., MINZ D., MULLER T., YERMIYAHU U., 2015 - *The use of biostimulants for enhancing nutrient uptake*. - Advances in Agronomy, Elsevier, pp. 141-174.

HAMOUDA A., HENDI D.M., ABU-EL-LEEL O., 2012 - *Improving basil growth, yield and oil production by* Aloe vera *extract and active dry yeast*. - Egypt. J. Hortic., 39: 45-71.

HARKER F., MARSH K., YOUNG H., MURRAY S., GUNSON F., WALKER S., 2002 - *Sensory interpretation of instrumental measurements 2: sweet and acid taste of apple fruit*. - Postharvest Biol. Technol., 24: 241-250.

HARKER F.R., AMOS R.L., ECHEVERRÍAA G., GUNSON F.A., 2006 - *Influence of texture on taste: Insights gained during studies of hardness, juiciness, and sweetness of*

apple fruit. - J. Food Sci., 71: S77-S82.
HATANO K.-I., KANAZAWA K., TOMURA H., YAMATSU T., TSUNODA K.-I., KUBOTA K., 2016 - *Molasses melanoidin promotes copper uptake for radish sprouts: the potential for an accelerator of phytoextraction*. - Environ. Sci. Pollut. Res., 23: 17656-17663.
HEMALATHA M., SELVI D., VASANTHI D., 2018 a. - *Integrated plant nutrients supply and foliar nutrition on fruit yield, nutrient uptake and availability in okra [*Abelmoschus esculentus *(L.) Moench] hybrid COBhH-4 in an alfisol of tamirabarani command area*. - Int. J. Adv. Agric. Sci. Tecnol., 5: 47-66.
HEMALATHA M., SELVI D., VASANTHI D., 2018 b - *Integrated plant nutrients supply and foliar nutrition on yield and quality of Okra [*Abelmoschus esculentus *(L.) Moench] hybrid COBhH-4 in Alfisols*. - Madras Agric. J., 105.
HENDI D.M., 2021 - *Utilization of* Aloe vera *gel as growth enhancer on micropropagation of* Eucalyptus citriodora *hook plant*. - Sci. J. Flowers Ornam. Plants, 8: 55-63.
HIRAKAWA H., SHIRASAWA K., KOSUGI S., TASHIRO K., NAKAYAMA S., YAMADA M., KOHARA M., WATANABE A., KISHIDA Y., FUJISHIRO T., 2014 - *Dissection of the octoploid strawberry genome by deep sequencing of the genomes of* Fragaria *species*. - DNA Res., 21: 169-181.
HOLCROFT D.M., KADER A.A., 1999 - *Controlled atmosphere-induced changes in pH and organic acid metabolism may affect color of stored strawberry fruit*. - Postharvest Biol. Technol., 17: 19-32.
ISHARTATI E., MEJAYA M., ZAKIA A., MARDIANTO R., 2019 - *The study of effectiveness root-up types and concentrations on root growth rate of potato (*Solanum tuberosum *L.) derived from cutting bud*. - IOP Conference Series: Earth and Environmental Science. IOP Publishing, pp. 012012.
ISHIMARU Y., KAKEI Y., SHIMO H., BASHIR K., SATO Y., SATO Y., UOZUMI N., NAKANISHI H., NISHIZAWA N.K., 2011 - *A rice phenolic efflux transporter is essential for solubilizing precipitated apoplasmic iron in the plant stele*. - J. Biol. Chem., 286: 24649-24655.
JAEGER S.R., MACHÍN L., ASCHEMANN-WITZEL J., ANTÚNEZ L., HARKER F.R., ARES G., 2018 - *Buy, eat or discard? A case study with apples to explore fruit quality perception and food waste*. - Food Qual. Prefer., 69: 10-20.
JOUQUAND C., CHANDLER C., PLOTTO A., GOODNER K., 2008 - *A sensory and chemical analysis of fresh strawberries over harvest dates and seasons reveals factors that affect eating quality*. - J. Am. Soc. Hortic. Sci., 133: 859-867.
KADIR S., CAREY E., ENNAHLI S., 2006 - *Influence of high tunnel and field conditions on strawberry growth and development*. - HortScience, 41: 329-335.
KHATER R.M., ABD-ALLAH W.H., EL SHAFAY R., 2020 - *Effect of organic fertilization and spraying* Aloe vera *extract on the growth and productivity of* Carum carvi *l. plant under shalateen conditions in Egypt*. - Plant Arch., 20: 4959-4971.
KHOO H.E., AZLAN A., TANG S.T., LIM S.M., 2017 - *Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits*. - Food Nutr. Res., 61: 1361779.
KLEE H.J., 2010 - *Improving the flavor of fresh fruits: genomics, biochemistry, and biotechnology*. - New Phytol., 187: 44-56.
KOŹMIŃSKA A., HANUS-FAJERSKA E., HALECKI W., CIARKOWSKA K., 2021 - *Beet molasses enhance salinity tolerance in* Thymus serpyllum - *a study under greenhouse condition*. - Plants, 10: 1819.
LEWERS K.S., NEWELL M.J., PARK E., LUO Y., 2020 - *Consumer preference and physiochemical analyses of fresh strawberries from ten cultivars*. - Int. J. Fruit Sci., 20: 733-756.
LI S., ZHAO X., YE X., ZHANG L., SHI L., XU F., DING G., 2020 - *The effects of condensed molasses soluble on the growth and development of rapeseed through seed germination, hydroponics and field trials*. - Agriculture, 10: 260.
LINGEGOWDA D.C., KUMAR J.K., PRASAD A.D., ZAREI, M., GOPAL S., 2012 - *FTIR spectroscopic studies on* Cleome gynandra - *comparative analysis of functional group before and after extraction*. - Rom. J. Biophys., 22: 137-143.
LINSTROM P.J., MALLARD W.G., 2001 - *The NIST chemistry WebBook: A chemical data resource on the internet*. - J. Chem. Eng. Data, 46: 1059-1063.
LOBELL D.B., GOURDJI S.M., 2012 - *The influence of climate change on global crop productivity*. - Plant Physiol., 160: 1686-1697.
MABRY T.J., MARKHAM K.R., THOMAS M.B., 1970 -. *The systematic identification of flavonoids*. - Springer-Verlag, New York.
MAGALHÃES L.M., SEGUNDO M.A., REIS S., LIMA J.L., 2008 - *Methodological aspects about* in vitro *evaluation of antioxidant properties*. - Anal. Chim. Acta, 613: 1-19.
MIRABELLA M., CUCINIELLO A., MAIELLO R., CENVINZO V., LEONE V., CARUSO G., 2021 - *Biostimulant application improves tomato (*Solanum lycopersicum *L.) fruit yield and quality during the autumn-winter season*. - Res. J. Agric. Sci., 53: 4.
MORAGA G., MARTÍNEZ-NAVARRETE N., CHIRALT A., 2006 - *Compositional changes of strawberry due to dehydration, cold storage and freezing-thawing processes*. - J. Food Process. Preserv., 30: 458-474.
NADEEKA P.W., SERAN T.H., 2020 - *The effects of goat manure and sugarcane molasses on the growth and yield of beetroot (*Beta vulgaris *L.)*. - J. Agric. Sci. (Belgr.), 65: 321-335.
NAGY A., MAGYAR T., JUHÁSZ C., TAMÁS J., 2020 - *Phytoremediation of acid mine drainage using by-product of lysine fermentation*. - Water Sci. Technol., 81:

1507-1517.

NAJAFPOUR G.D., SHAN C.P., *2003 - Enzymatic hydrolysis of molasses*. - Bioresour. Technol., 86: 91-94.

NIST, 2022 - *Water*. - National Institut of Standard and Technology, NIST, Chemistry WebBook, SRD 69.

PARAĐIKOVIĆ N., TEKLIĆ T., ZELJKOVIĆ S., LISJAK M., ŠPOLJAREVIĆ M., 2019 - *Biostimulants research in some horticultural plant species - A review*. - Food Energy Secur., 8: e00162.

PLOTTO A., WHITAKER V., CHANDLER C., 2010 - *Eating quality of old and new University of Florida strawberry cultivars*. - Proceed. Florida State Hortic. Soc., pp. 290-295.

POLING E.B., 2012 - *Strawberry plant structure and growth habit*. - New York State Berry Growers Association, Berry EXPO.

PYAKUREL A., DAHAL B.R., RIJAL S., 2019 - *Effect of molasses and organic fertilizer in soil fertility and yield of spinach in khotang, Nepal*. - Int. J. Appl. Sci. Biotechnol., 7: 49-53.

RAHARJO B., TRANGGONO T., DETHAN J., 1998 - *Textural behavior of ripening sapota fruits*. Indones. - Food Nutr. Prog., 5: 43-48.

RAHMAN M., RAHMAN M., ISLAM T., 2019 - *Improving yield and antioxidant properties of strawberries by utilizing microbes and natural products.* - In: ASAO T., and M. ASADUZZAMAN (eds.) *Strawberry pre- and post-harvest management techniques for higher fruit quality.* IntechOpen, London, UK, pp. 156

RICHTER H., 1978 - *A diagram for the description of water relations in plant cells and organs*. - J. Exp. Bot., 29: 1197-1203.

RODRIGUES M., BAPTISTELLA J.L.C., HORZ D.C., BORTOLATO L.M., MAZZAFERA P., 2020 - *Organic plant biostimulants and fruit quality - A review*. Agron., 10: 988.

ROGER HARKER F., LAU K., ANNE GUNSON F., 2003 - *Juiciness of fresh fruit: a time-intensity study*. - Postharvest Biol. Technol., 29: 55-60.

ROUPHAEL Y., COLLA G., GIORDANO M., EL-NAKHEL C., KYRIACOU M.C., DE PASCALE S., 2017 - *Foliar applications of a legume-derived protein hydrolysate elicit dose-dependent increases of growth, leaf mineral composition, yield and fruit quality in two greenhouse tomato cultivars*. - Sci. Hortic., 226: 353-360.

SALENTIJN E.M., AHARONI A., SCHAART J.G., BOONE M.J., KRENS F.A., 2003 - *Differential gene expression analysis of strawberry cultivars that differ in fruit-firmness*. - Physiol. Plant., 118: 571-578.

SALIBA-COLOMBANI V., CAUSSE M., LANGLOIS D., PHILOUZE J., BURET M., 2001 - *Genetic analysis of organoleptic quality in fresh market tomato. 1. Mapping QTLs for physical and chemical traits*. - Theor. Appl. Genet., 102: 259-272.

SARANWONG I., SORNSRIVICHAI J., KAWANO S., 2003 - *Performance of a portable near infrared instrument for Brix value determination of intact mango fruit*. - J. Near Infrared Spectrosc., 11: 175-181.

SAVINI G., NERI D., ZUCCONI F., SUGIYAMA N., 2005 - *Strawberry growth and flowering: an architectural model*. - Int. J. Fruit Sci., 5: 29-50.

SCHNEIDER C.A., RASBAND W.S., ELICEIRI K.W., 2012 - *NIH Image to ImageJ: 25 years of image analysis*. - Nat. Methods, 9: 671-675.

SELBY-PHAM S.N.B., COTTRELL J., DUNSHEA F., NG K., BENNETT L.E., HOWELL K., 2017 - *Dietary phytochemicals promote health by enhancing antioxidant defence in a pig model*. - Nutrients, 9: 758.

SHAHZAD H., IQBAL M., BASHIR S., FAROOQ M., 2018 - *Relative efficacy of organic substrates on maize root proliferation under water stress*. - Biosci. J., 35.

SRIVASTAVA P., SINGH R., SRIVASTAVA P., SHRIVASTAVA M., 2012 - *Utilization of molasses based distillery effluent for fertigation of sugarcane*. - Biodegradation, 23: 897-905.

STRIK B.C., PROCTOR J.T., 1988 - *Yield component analysis of strawberry genotypes differing in productivity*. - J. Am. Soc. Hortic. Sci., 113: 124-129.

SULIASIH S., WIDAWATI S., 2016 - *Effect of plant growth promoting rhizobacteria and molasses on seed germination and seedling growth of* Sorghum bicolor *l. Moench*. - The 1st SATREPS Conference, Bogor, Indonesia.

SZCZESNIAK A.S., SMITH B.J., 1969 - *Observations on strawberry texture a three-pronged approach*. - J. Texture Stud., 1: 65-89.

THAKARE P., CHAUDHARY M., POKAL W., 2013 - *Physico-chemical characterisation of molasses and its effects on the growth of* Abelmoschus esculentus *(Lady's Finger)*. - World Appl. Sci. J., 21: 869-872.

TIEMAN D., ZHU G., RESENDE M.F., LIN T., NGUYEN C., BIES D., RAMBLA J.L., BELTRAN K.S.O., TAYLOR M., ZHANG B., 2017 - *A chemical genetic roadmap to improved tomato flavor*. - Science, 355: 391-394.

TOMIC J., PESAKOVIC M., MILIVOJEVIC J., MILETIC R., KARAKLAJIC-STAJIC Z., PAUNOVIC S., MILINKOVIC M., 2016 - *Changes in anthocyanins and total phenols in fruit of three strawberry cultivars during five harvests*. - Acta Horticulturae, 1139: 633-638.

TOPOLSKA K., FLORKIEWICZ A., FILIPIAK-FLORKIEWICZ A., 2021 - *Functional food - Consumer motivations and expectations*. - Int. J. Environ. Res. Public Health, 18: 5327.

UDDIN A., RAKIBUZZAMAN M., RAISA I., MALIHA M., HUSNA M., 2020 - *Impact of natural substances and synthetic hormone on grapevine cutting*. - J. Biosci. Agric. Res., 25: 2069-2074.

USDA, 2006 - *United states standards for grades of strawberries*. - USDA, US Department of Agriculture. https://www.ams.usda.gov/grades-standards/strawberries-grades-and-standards.

WAHYUNINGSIH S., WULANDARI L., WARTONO M., MUNAWAROH H., RAMELAN A., 2017 - *The effect of pH*

and color stability of anthocyanin on food colorant. - IOP conference series: Materials science and engineering. IOP Publishing, pp. 012047.

WANG J., YUE C., GALLARDO K., MCCRACKEN V., LUBY J., MCFERSON J., 2017 - *What consumers are looking for in strawberries: Implications from market segmentation analysis*. - J. Agribus., 33: 56-69.

WANG P., YANG J., LI Z.-Y., ZHU J.-J., GAO Q.-H., NI D.-A., DUAN K., 2021 - *Genome-wide identification and expression analysis revealed cinnamyl alcohol dehydrogenase genes correlated with fruit-firmness in strawberry*. - J. Berry Res., 1-18.

WANG Z., ZHANG M., WU Q., 2015 - *Effects of temperature, pH, and sunlight exposure on the color stability of strawberry juice during processing and storage*. - LWT - Food Sci. Technol., 60: 1174-1178.

WELBAUM G.E., STURZ A.V., DONG Z., NOWAK J., 2004 - *Managing soil microorganisms to improve productivity of agro-ecosystems*. - Crit. Rev. Plant Sci., 23: 175-193.

WENDIN K., EGAN P., OLSSON V., FORSBERG S., NILSSON A., STENBERG J., 2019 - *Is there a best woodland strawberry? A consumer survey of preferred sensory properties and cultivation characteristics*. - Int. J. Gastron. Food Sci., 16: 100151.

WISE K., GILL H., SELBY-PHAM J., 2020 - *Willow bark extract and the biostimulant complex Root Nectar® increase propagation efficiency in chrysanthemum and lavender cuttings*. - Sci. Horticult., 263: 109108.

WISE K., WEDDING T., SELBY-PHAM J., 2022 - *Application of automated image colour analyses for the early-prediction of strawberry development and quality*. - Sci. Horticult., 304: 111316.

WOOLWORTHS SUPERMARKETS LTD, 2010 - *Product Specifications Strawberry*. - Product Specifications: Berries. https://www.gfap.com.au/link/application/archive/yv/upload/2971/Berries%20specifications%20-%20Woolworths%2030-7-10.pdf

WOZNIAK W., RADAJEWSKA B., RESZELSKA-SIECIECHOWICZ A., DEJWOR I., 1996 - *Sugars and acid content influence organoleptic evaluation of fruits of six strawberry cultivars from controlled cultivation*. - Acta Horticulturae, 439: 333-336.

YAN J.W., BAN Z.J., LU H.Y., LI D., POVERENOV E., LUO Z.S., LI L., 2018 - *The aroma volatile repertoire in strawberry fruit: a review*. - J. Sci. Food Agric., 98: 4395-4402.

YOSHIDA Y., KOYAMA N., TAMURA H., 2002 - *Color and anthocyanin composition of strawberry fruit: Changes during fruit development and differences among cultivars, with special reference to the occurrence of pelargonidin 3-malonylglucoside*. - J. Jpn. Soc. Hortic. Sci., 71: 355-361.

ZHANG D., HAMAUZU Y., 2004 - *Phenolic compounds and their antioxidant properties in different tissues of carrots (*Daucus carota *L.)*. - J. Food Agric. Environ., 2: 95-100.

ZHANG Y., SEERAM N.P., LEE R., FENG L., HEBER D., 2008 - *Isolation and identification of strawberry phenolics with antioxidant and human cancer cell antiproliferative properties*. - J. Agric. Food Chem., 56: 670-675.

ZHOU W., ZHENG W., LV H., WANG Q., LIANG B., LI J., 2022 - *Foliar application of pig blood-derived protein hydrolysates improves antioxidant activities in lettuce by regulating phenolic biosynthesis without compromising yield production*. - Sci. Horticult., 291: 110602.