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

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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 × ananassa*, is the most popular variety, accounting for 60% of the world’s 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 as 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 (melanoids) (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 (Nitriﬁeld 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% v 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)

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Fertigation programme (split evenly throughout the day)</th>
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<tr>
<td>6-11</td>
<td>3 × 10 min</td>
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<tr>
<td>12-13</td>
<td>4 × 10 min</td>
</tr>
<tr>
<td>14-18</td>
<td>6 × 10 min</td>
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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) saturated 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>
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<th>Sensory perception scoring matrix</th>
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<tr>
<td><strong>Aroma - Desirability</strong></td>
</tr>
<tr>
<td>Poor</td>
</tr>
<tr>
<td>Acceptor</td>
</tr>
<tr>
<td><strong>Mouthfeel - Firmness</strong></td>
</tr>
<tr>
<td>Low</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td><strong>Mouthfeel - Juiciness</strong></td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td><strong>Aroma - Intensity</strong></td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td><strong>Taste - Sweet</strong></td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td><strong>Taste - Sour/acid</strong></td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Moderate</td>
</tr>
<tr>
<td><strong>Taste - Intensity</strong></td>
</tr>
<tr>
<td>None</td>
</tr>
<tr>
<td>Moderate</td>
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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 (cm²), 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·g⁻¹ d.w.), Brix %, fruit pH, and fruit water % (Q1, Q2, Q3, and Q5, respectively).

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 cm² in control to 609.3 cm² 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. 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.

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 $p ≤ 0.001$ calculated by Student’s t-test.
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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·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), (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 ≤ 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 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 (Szczesiak 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 (Szczesiak 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 alky-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 flavlyium 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, vegetative 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.

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