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Supplementary materials

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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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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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.

Table S1 - Nutrient profile of biostimulant comples (BC)

Nutrient	Content (mg· L^{-1})
Nitrogen[A]	2600
Phosphorus[B]	454.25
Potassium[B]	10230.5
Sulfur[A]	4400
Calcium[B]	592.5
Magnesium[B]	2133
Silica (SiO_2) [B]	211.3
Boron[B]	<50
Iron[B]	<50
Zinc[B]	<50
Manganese[B]	<50

A = Quantified via LECO TruMac CNS Analyser as per Rayment, G.E., Lyons, D.J., 2011. Soil chemical methods: Australasia. CSIRO publishing.
B = ICP/AES by ACS Laboratories, Victoria, Australia.

Table S2 - Phytohormone profile of biostimulant complex (BC) and reservoir solutions

Phytohormone	BC (ng · ml^{-1})	BC reservoir (ng · ml^{-1})	Control reservoir (ng · ml^{-1})
Gibberellin A3	0.000	0.000	0.000
Gibberellin A4	0.000	0.000	0.000
Indole-3-acetic acid (IAA)	0.000	0.000	0.000
Methyl-IAA	0.000	0.000	0.000
Indole-3-carboxylic acid (ICA)	0.000	0.000	0.000
Zeatin (cytokinin)	70.362	5.251	0.000
Abscisic acid (ABA)	121.043	0.000	0.000
Jasmonic acid (JA)	15.824	0.000	0.000
Methyl-JA	0.000	0.000	0.000
JA-Isoleucine	4.077	0.000	0.000
12-Oxo-phytodienoic acid (OPDA)	42.937	0.000	0.000
Salicylic acid	822.589	0.000	0.000
Cinnamic acid	123.408	0.000	0.000
Brassinolide	0.000	0.000	0.000

Phytohormone quntification performed as per: Suwanchaikasem P., Idnurm A., Selby-Pham J., Walker R., Boughton B.A., 2022 - *Root-TRAPR: a modular plant growth device to visualize root development and monitor growth parameters, as applied to an elicitor response of* Cannabis sativa. - Plant methods, 18(1): 1-20.

Table S3 a - Metabolic profile of biostimulant complex (BC)

Name	Formula	Molecular weight	RT [min]	Peak area
(-)-Spiculisporic acid	C17 H28 O6	328.189	31.781	187.48
(+)-streptol	C7 H12 O5	1.760.684	3.246	2.64
(15Z)-9,12,13-Trihydroxy-15-octadecenoic acid	C18 H34 O5	33.024.104	29.362	32.94
(1S,3R,4R,5R)-1,3,4-trihydroxy-5-{[(2E)-3-(4-hydroxy-3-methoxyphenyl)prop-2-enoyl]oxy}cyclohexane-1-carboxylic acid	C17 H20 O9	36.811.126	24.763	45.92
(2R)-2-Hydroxy-3-[(hydroxy{[(1S,2R,3R,4S,5S,6R)-2,3,4,5,6-pentahydroxycyclohexyl]oxy}phosphoryl)oxy]propyl palmitate	C25 H49 O12 P	5.722.965	32.936	38.69
(2R,3S)-3-isopropylmalic acid	C7 H12 O5	17.606.842	3.092	1.34
(3R,5R)-1,3,5-Trihydroxy-4-{[(2E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoyl]oxy}cyclohexanecarboxylic acid	C17 H20 O9	36.811.125	19.92	54.62
(3R,5R)-1,3,5-Trihydroxy-4-{[(2E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoyl]oxy}cyclohexanecarboxylic acid	C17 H20 O9	36.811.129	24.335	36.45
(3S,4S)-3-Hydroxy-1,3,4-tetradecanetricarboxylic acid	C17 H30 O7	34.619.955	31.211	28.56
(DL)-3-O-Methyldopa	C10 H13 N O4	21.108.455	17.674	9.67
[7-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-3-(hydroxymethyl)-6-methoxy-1,2,3,4-tetrahydro-2-naphthalenyl]methyl pentofuranoside	C25 H32 O10	49.220.024	26.447	1.22
1-(2-Deoxypentofuranosyl)-5-methyl-2,4(1H,3H)-pyrimidinedione	C10 H14 N2 O5	24.209.055	2.376	0.73
1-(3-Acetyl-2,4,6-trihydroxyphenyl)-1,5-anhydrohexitol	C14 H18 O9	33.009.558	10.077	42.08
1-(6-hydroxy-3-pyridyl)-4-(methylamino)butan-1-one	C10 H14 N2 O3	21.010.054	24.059	0.34
1,5-Anhydro-1-[5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-4-oxo-4H-chromen-8-yl]hexitol	C23 H24 O11	47.613.266	26.635	14.63
1-oleoylglycerone 3-phosphate	C21 H39 O7 P	43.424.396	33.182	56.83
1-Palmitoyl lysophosphatidic acid	C19 H39 O7 P	41.024.397	33.428	39.08
2-(2,4-Dihydroxyphenyl)-5,7-dihydroxy-6-(3-hydroxy-3-methylbutyl)-4H-chromen-4-one	C20 H20 O7	37.212.146	28.055	7.63
2-(Acetylamino)hexanoic acid	C8 H15 N O3	17.310.509	16.179	3.41
2-(Acetylamino)hexanoic acid	C8 H15 N O3	17.310.508	14.583	3.37
2,3-Dihydro-1-benzofuran-2-carboxylic acid	C9 H8 O3	16.404.707	23.039	67.63
2,5-Dihydroxybenzaldehyde	C7 H6 O3	13.803.173	7.814	40.62
2-[7,8-Dihydroxy-6-(hydroxymethyl)-2-methylhexahydro-4aH-pyrano[2,3-b][1,4]dioxin-2-yl]-5-methylcyclohexanone	C16 H26 O7	3.301.682	31.454	15.07
2-Hydroxy-3-[3-(3-methyl-2-buten-1-yl)-4-(sulfooxy)phenyl]propanoic acid	C14 H18 O7 S	33.007.778	25.808	0.45
2-Hydroxycaproic acid	C6 H12 O3	13.207.844	8.296	48.17
2-Hydroxycaproic acid	C6 H12 O3	13.207.842	9.409	38.20
2-hydroxysebacic acid	C10 H18 O5	21.811.568	24.571	9.83
2-Isopropylmalic acid	C7 H12 O5	17.606.851	7.107	36.51
2-Methyl-3-hydroxybutyric acid	C5 H10 O3	1.180.628	3.327	8.95
2-O-caffeoylglucaric acid	C15 H16 O11	3.720.696	24.385	28.00
3-(4-Hydroxy-3,5-dimethoxyphenyl)-2-oxiranecarboxylic acid	C11 H12 O6	24.006.387	3.167	0.10
3-(6,7-Dimethoxy-1,3-benzodioxol-5-yl)-2-oxiranecarbaldehyde	C12 H12 O6	25.206.357	25.767	0.14
3-(6,7-Dimethoxy-1,3-benzodioxol-5-yl)-2-propen-1-ol	C12 H14 O5	2.380.843	24.242	12.62

Secondary metabolite profiling performed using Reverse Phase (RP) HPLC-HRFTMS by The Australian Wine Research Institute (AWRI).

Table S3 b - Metabolic profile of biostimulant complex (BC)

Name	Formula	Molecular weight	RT [min]	Peak area
3,5-Dihydroxy-2-[3-(3-hydroxy-4-methoxyphenyl)propanoyl]phenyl hexopyranosiduronic acid	C22 H24 O12	48.012.732	26.843	13.86
3-[3-(beta-D-Glucopyranosyloxy)-2-hydroxyphenyl]propanoic acid	C15 H20 O9	3.441.112	14.876	13.61
3-Methoxy-4-hydroxyphenylglycol glucuronide	C15 H20 O10	36.010.629	7.82	31.24
3-O-methylgallic acid	C8 H8 O5	18.403.706	12.221	120.37
3-Phenyllactic acid	C9 H10 O3	1.660.628	14.906	27.57
4-(2-Carboxyethyl)-2-methoxyphenyl beta-D-glucopyranosiduronic acid	C16 H20 O10	37.210.607	22.831	85.21
4-(2-Carboxyethyl)-2-methoxyphenyl beta-D-glucopyranosiduronic acid	C16 H20 O10	37.210.594	17.893	10.81
4-Hydroxybenzaldehyde	C7 H6 O2	1.220.367	11.398	38.26
4-Oxoproline	C5 H7 N O3	1.290.424	1.261	68.37
4-Oxoproline	C5 H7 N O3	12.904.227	1.478	0.18
5-(3',5'-Dihydroxyphenyl)-gamma-valerolactone 3-O-glucuronide	C17 H20 O10	38.410.614	24.376	24.80
6-O-sinapoyl-D-glucono-1,5-lactone	C17 H20 O10	38.410.631	15.108	7.78
Arachidonic acid	C20 H32 O2	30.424.055	33.885	0.03
asp-leu	C10 H18 N2 O5	24.612.169	4.442	4.61
Azelaic acid	C9 H16 O4	18.810.508	25.675	16.38
Caffeic acid	C9 H8 O4	18.004.246	17.113	14.00
Catalpol	C15 H22 O10	36.212.169	12.848	153.83
Chelidonic acid	C7 H4 O6	18.400.088	1.34	4.34
Chlorogenic acid	C16 H18 O9	35.409.543	20.664	130.00
Choline O-Sulfate	C5 H13 N O4 S	18.305.659	0.934	2.18
Citraconic acid	C5 H6 O4	13.002.652	1.151	49.63
Citric acid	C6 H8 O7	19.202.657	1.151	2023.75
Corchorifatty acid F	C18 H32 O5	32.822.539	28.816	12.55
D-(-)-Fructose	C6 H12 O6	18.006.339	1.369	60.40
D-(-)-Fructose	C6 H12 O6	12.004.293	1.128	16.65
D-(-)-Quinic acid	C7 H12 O6	19.206.351	0.945	18.95
DL-4-Hydroxyphenyllactic acid	C9 H10 O4	18.205.822	7.4	11.00
DL-Malic acid	C4 H6 O5	13.402.141	1.016	211.49
D-Xylonic acid	C5 H10 O6	16.604.796	0.929	119.88
D-α-Hydroxyglutaric acid	C5 H8 O5	14.803.703	1.227	54.09
D-α-Hydroxyglutaric acid	C5 H8 O5	14.803.673	1.399	24.65
Eicosapentaenoic acid	C20 H30 O2	30.222.489	33.652	0.01
Fertaric acid	C14 H14 O9	3.260.644	25.81	13.23
Flazin	C17 H12 N2 O4	30.808.017	28.778	10.77
Gallic acid	C7 H6 O5	17.002.136	3.018	806.09
Gentisic acid	C7 H6 O4	15.402.653	5.435	1.52

Secondary metabolite profiling performed using Reverse Phase (RP) HPLC-HRFTMS by The Australian Wine Research Institute (AWRI).

Table S3 c - Metabolic profile of biostimulant complex (BC)

Name	Formula	Molecular Weight	RT [min]	Peak area
Î²-D-Glucopyranuronic acid, 1-(3-methylbutanoate)	C11 H18 O8	27.810.045	6.057	18.49
Imidazoleacetic acid riboside	C10 H14 N2 O6	25.808.548	2.102	5.37
Leu-Val	C11 H22 N2 O3	23.016.357	10.268	13.06
Leu-Val	C11 H22 N2 O3	2.301.634	7.103	6.52
L-Phenylalanine	C9 H11 N O2	16.507.908	2.921	2.52
Malonic acid	C3 H4 O4	10.401.092	1.057	16.78
Malonic acid	C3 H4 O4	1.040.109	1.107	5.35
Methylsuccinic acid	C5 H8 O4	13.204.228	3.259	17.43
MX5800000	C8 H10 O4	17.005.807	4.75	1.61
Myristylsulfate	C14 H30 O4 S	29.418.675	31.153	427.93
N-(3,4-Dihydroxyphenyl)glutamine	C11 H14 N2 O5	25.409.043	25.249	12.57
N-[(2S)-2-Hydroxypropanoyl]-L-tryptophan	C14 H16 N2 O4	2.761.112	22.946	19.67
N-Acetyl-L-methionine	C7 H13 N O3 S	19.106.168	7.388	0.05
N-Acetyl-L-phenylalanine	C11 H13 N O3	20.708.982	21.533	2.78
Neochlorogenic acid	C16 H18 O9	3.540.955	10.497	118.55
Pantothenic acid	C9 H17 N O5	21.911.094	4.822	42.68
Phaseolic acid	C13 H12 O8	29.605.366	24.186	19.41
Pimelic acid	C7 H12 O4	16.007.348	5.721	2.86
Pimelic acid	C7 H12 O4	16.007.346	5.256	2.37
porphobilinogen	C10 H14 N2 O4	22.609.631	7.798	8.79
Quercetin	C15 H10 O7	30.204.315	28.599	0.08
Questiomycin A	C12 H8 N2 O2	21.205.878	23.037	3.53
Saccharin	C7 H5 N O3 S	18.299.877	3.44	12.27
Sakuranin	C22 H24 O10	44.813.762	28.211	6.45
Salicylic acid	C7 H6 O3	13.803.172	21.923	31.37
Seryltyrosine	C12 H16 N2 O5	26.810.603	26.872	13.50
shanzhiside	C16 H24 O11	39.213.231	17.69	79.16
Suberic acid	C8 H14 O4	17.408.925	2.973	1.18
Sucrose	C12 H22 O11	34.211.672	0.949	25.06
Swertiajaponin	C22 H22 O11	46.211.704	25.624	31.75
Trifluoroacetic acid	C2 H F3 O2	11.399.281	1.096	3.80
Valylvaline	C10 H20 N2 O3	21.614.764	2.764	1.75
Vanillin	C8 H8 O3	15.204.728	13.382	18.82
Volkenin	C12 H17 N O7	28.710.087	23.619	1.61

Secondary metabolite profiling performed using Reverse Phase (RP) HPLC-HRFTMS by The Australian Wine Research Institute (AWRI).

Table 4 - Impact of the biostimulant complex (BC) on strawberry growth, yield, and quality

Measure	Average value		P
	Control	BC	
Total fruit harvested (number · plant^{-1})	12.00 (n = 6)	18.83 (n = 6)	0.035
Total fruit harvested (g · plant^{-1})	128.3 (n = 6)	193.3 (n = 6)	0.038
Total unripe fruit (number · plant)	10.33 (n = 6)	11.17 (n = 6)	0.814
Total unripe fruit (g · plant^{-1})	35.18 (n = 6)	27.53 (n = 6)	0.600
Number of crowns	3.333 (n = 6)	4.500 (n = 6)	0.057
Number of leaves	15.67 (n = 6)	32.50 (n = 6)	< 0.001
Leaf fresh weight (g f.w.)	28.49 (n = 6)	50.46 (n = 6)	< 0.001
Leaf dry weight (g d.w.)	7.408 (n = 6)	12.350 (n = 6)	0.002
Leaf water %	74.210 (n = 6)	75.584 (n = 6)	0.205
Aerial (non-leaf) fresh weight (g f.w.)	29.08 (n = 6)	50.46 (n = 6)	< 0.001
Aerial (non-leaf) dry weight (g d.w.)	5.717 (n = 6)	9.383 (n = 6)	0.001
Canopy area (cm^2)	401.4 (n = 6)	609.3 (n = 6)	0.001
Leaf colour (*L**)	24.977 (n = 6)	25.370 (n = 6)	0.390
Leaf colour (*a**)	-4.978 (n = 6)	-4.908 (n = 6)	0.829
Leaf colour (*b**)	5.362 (n = 6)	5.275 (n = 6)	0.812
Brix of crude extract (°Bx)	7.867 (n = 6)	7.831 (n = 13)	0.941
pH of crude extraxt (1/1000 dilution in water)	4.2383 (n = 6)	4.3531 (n = 13)	0.055
Fruit length (mm)	30.33 (n = 15)	35.48 (n = 21)	0.013
Fruit width (mm)	25.27 (n = 10)	26.50 (n = 12)	0.446
Fruit fresh weight (g f.w.)	10.691 (n = 72)	10.354 (n = 112)	0.666
Fruit quality compliance (% fruit count with FW > 10 g)	43.1 (n = 72)	44.6 (n = 112)	0.832
Fruit dry weight (g d.w.)	1.1193 (n = 22)	0.9317 (n = 27)	0.069
Fruit water %	89.061 (n = 22)	90.240 (n = 27)	0.001
Fruit total solids %	10.939 (n = 22)	9.760 (n = 27)	0.001
Fruit extract (g·g^{-1} d.w.)	7.126 (n = 22)	7.887 (n = 27)	0.068
Total polyphenolics (GAE g · g^{-1}d.w.)	0.01391 (n = 22)	0.01835 (n = 27)	0.029
Total flavonoids (QE mg · g^{-1}d.w.)	16.48 (n = 22)	17.80 (n = 27)	0.534
Harvest timing (days)	30.16 (n = 72)	30.17 (n = 97)	0.992
Fruit image colour A%	36.77 (n = 22)	33.92 (n = 27)	0.186
Fruit image colour O%	28.90 (n = 22)	20.79 (n = 27)	0.006
Fruit image colour G%	0.3831 (n = 22)	1.008 (n = 27)	0.001
Fruit image colour Y%	4.282 (n = 22)	5.061 (n = 27)	0.371
Fruit image colour R%	29.66 (n = 22)	39.22 (n = 27)	0.001

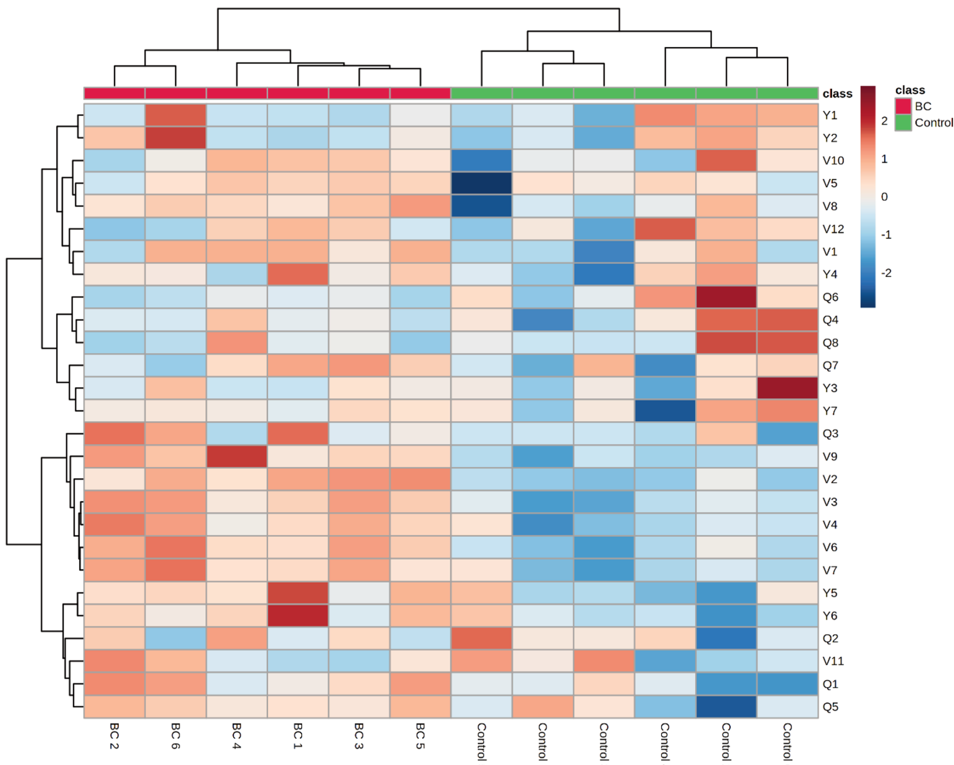

Fig. S1 - Heatmap of all vegetative, yield, and quality measures on strawberry plants

Table S5 - Measure code descriptors for heatmap (Fig. S1)

Code	Measure	Code	Measure
Q1	Total polyphenolics(GAE g · g^{-1}d.w.)	V7	Aerial (non-leaf) dry weight (g d.w.)
Q2	Brix of crude extract (°Bx)	V8	Aerial (non-leaf) water (%)
Q3	pH of crude extraxt (1/1000 dilution in water)	V9	Canopy area (cm^2)
Q4	Fruit dry weight (g d.w.)	V10	Leaf colour (*L**)
Q5	Fruit water %	V11	Leaf colour (*a**)
Q6	Fruit solids %	V12	Leaf colour (*b**)
Q7	Fruit extract (g·g^{-1} d.w.)	Y1	Unripe fruit (g)
Q8	Total flavonoids (QE mg · g^{-1}d.w.)	Y2	Total unripe fruit (number ·$plant^{-1}$)
V1	Number of crowns	Y3	Fruit fresh weight (g f.w.)
V2	Number of leaves	Y4	Harvest timing (days)
V3	Leaf fresh weight (gf.w.)	Y5	Total fruit harvested (g · $plant^{-1}$)
V4	Leaf dry weight (gd.w.)	Y6	Total fruit harvested (number · $plant^{-1}$)
V5	Leaf water (%)	Y7	Fruit quality compliance (% fruit count with FW > 10
V6	Aerial (non-leaf) fresh weight (g f.w.)		

Table S6 - Impact of BC on strawberry sensory perception

Measure	Average score		P
	Control	BC	
Aroma - Desirability	5.000	6.667	0.067
Taste - Desirability	4.500	5.170	0.555
Mouthfeel - Firmness	4.167	5.500	0.043
Mouthfeel – Juiciness	4.830	4.167	0.465
Aroma – Intensity	3.500	5.833	0.040
Taste - Sweet	4.000	3.833	0.822
Taste - Sour/acid	4.170	4.167	1.000
Taste - Intensity	4.670	4.500	0.899

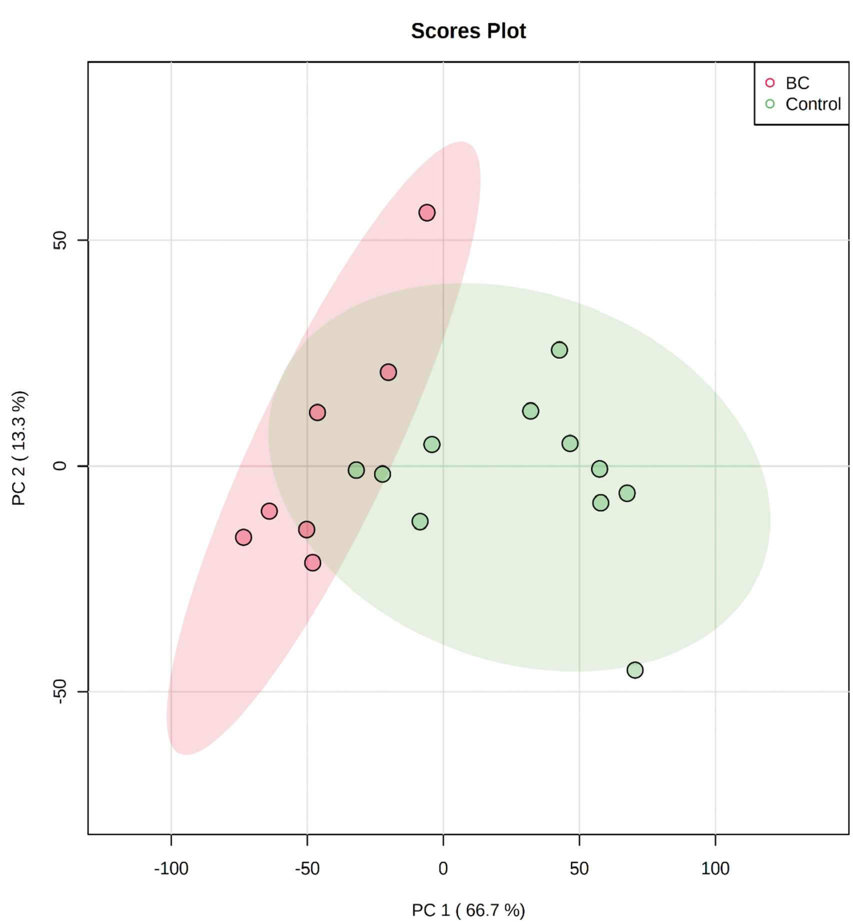

Fig. S2 - Principal component analysis (PCA) of M-IR spectra with shading indicating 95% confidence region.

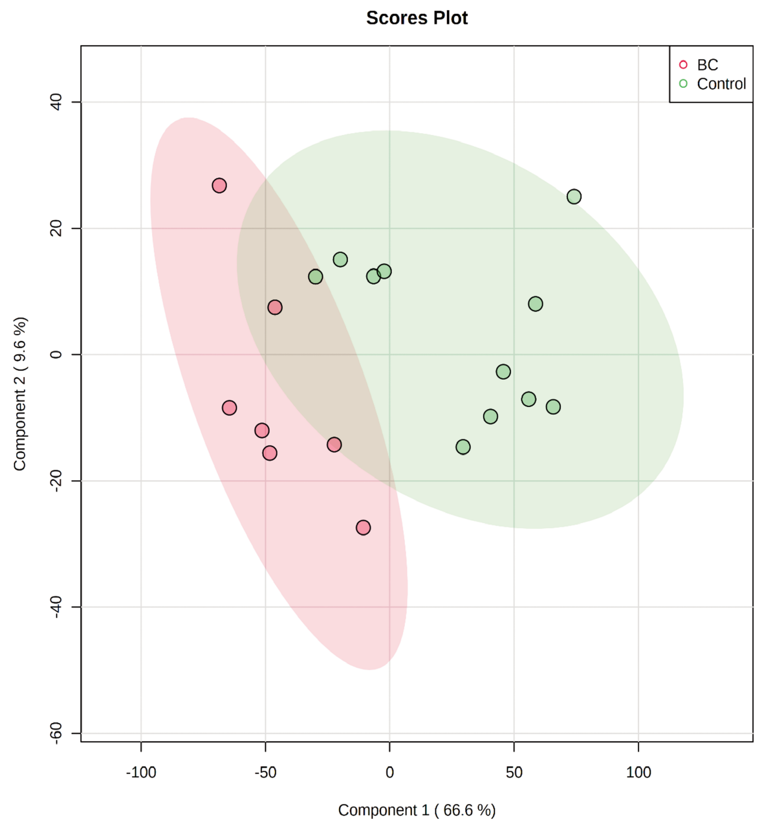

Fig. S3 - Partial least squares-discriminant analysis (PLS-DA) of M-IR spectra with shading indicating 95% confidence regions.

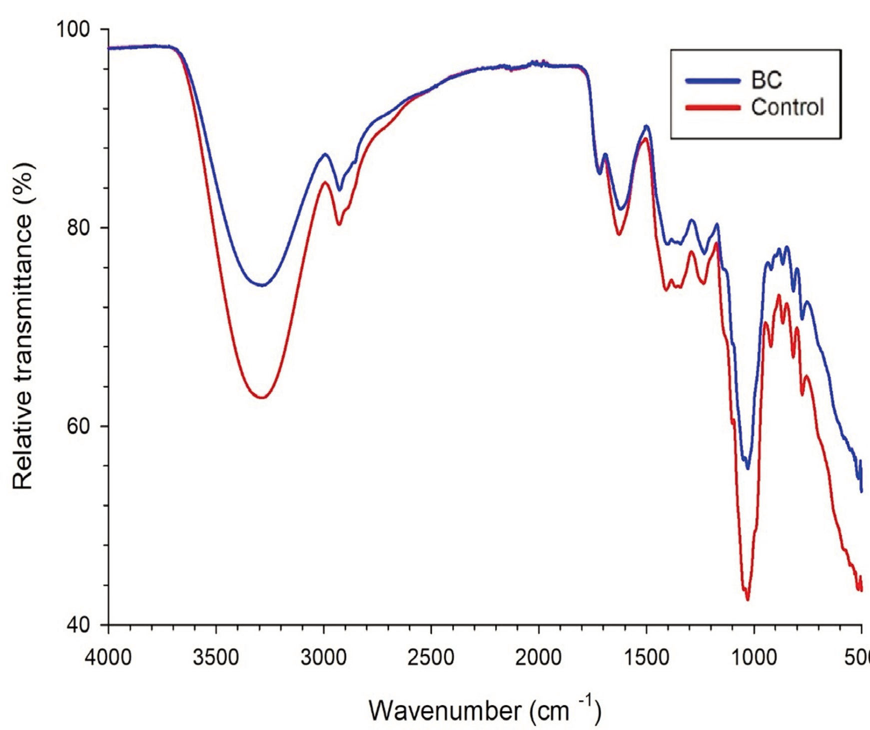

Fig. S4 - Mid-infrared (M-IR) spectra of dehydrated strawberry fruits analysed between 500–4000 cm-1 equipped with a Universal Attenuated Total Reflectance (UATR) accessory with diamond crystal. Spectra represent average absorbance of 7 and 11 biological replicates for control and BC, respectively.

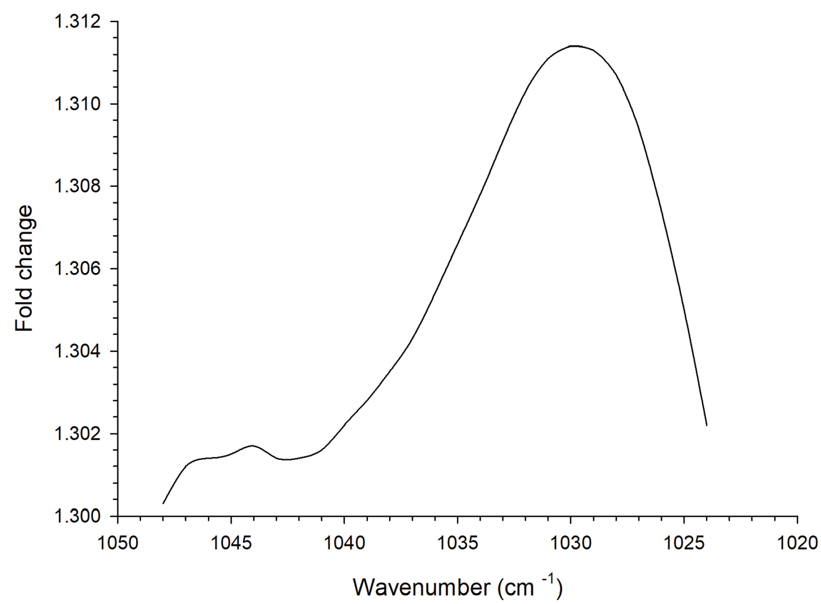

Fig. S5 - Fold change (> 1.3) in absorbance of average M-IR spectra for BC relative to average M-IRspectra for control.

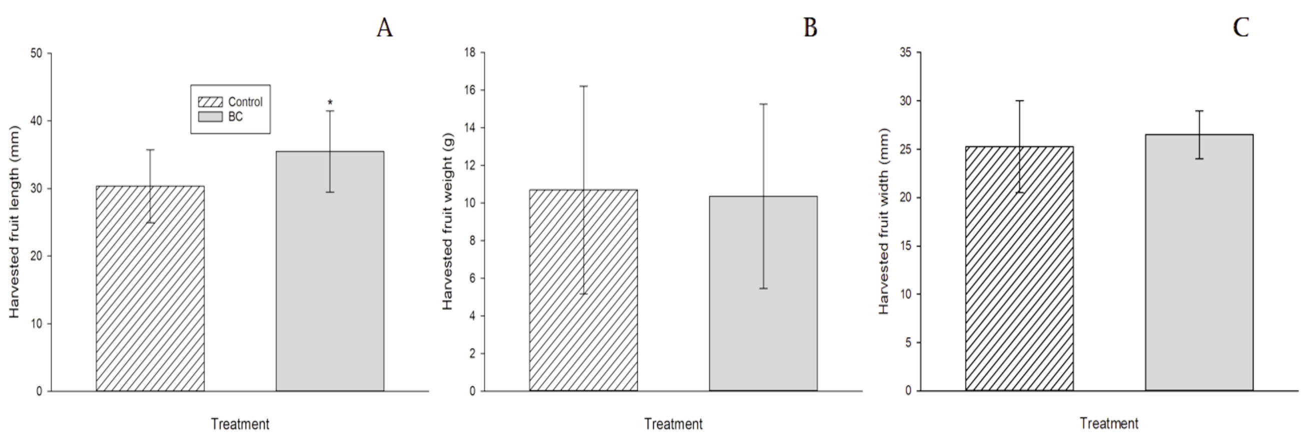

Fig. S6 - Effect of biostimulant complex (BC) on strawberry (*Fragaria x ananassa* 'Albion') fruit size parameters. A) length (control n = 15, BC n = 21), B) weight (control n = 10, BC = 12), and C) width (control n = 72, BC n = 112). Significant differences are indicated by * for p< 0.05 calculated by Student's t-test.