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*Original Research Paper*

# **Enhancement of tomato functional food value through nutrient supplementation with fish emulsion biostimulant**

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#### **ABSTRACT**

Fish emulsion (FE) is a derivative of fish waste, commonly utilised within organic agricultural and horticultural applications, predominantly as a source of nitrogen within fertiliser regimens. However, as a biological derivative, FE is a complex of many bioactive compounds, and as such is also known to function as a biostimulant. Tomato (*Solanum lycopersicum*) is a functional food, commonly produced for consumption due to its desirable hedonic qualities and health-promoting properties associated with antioxidant phytochemicals. Accordingly, the work herein explored the potential for FE to alter the functional and hedonic measures of tomato. Results indicated that the supplementation of fertiliser regimens with FE during tomato growth significantly ( $p = 0.001$ ) increased fruit total phenolic content by 1.25-fold, whilst not significantly impacting flavonoid content ( $p = 0.418$ ) or fruit colour (assessed by image colour analyses). Additionally, the FE treatment did not substantially impact sensory perception of hedonic measures such as smell, taste, mouth feel, or visual appeal. Accordingly, the results herein indicate that FE is a desirable fertiliser supplement during tomato cultivation, to enhance the functional value of tomato fruits, and thereby provide enhanced health-promoting benefits from tomato consumption.

**Keywords :** Antioxidant, fertiliser, fish hydrolysate, organic, *Solanum lycopersicum*

## **INTRODUCTION**

Tomato (*Solanum lycopersicum*) is one of the most popular food crops in the world (PA Silva et al., 2019), with 189.1 Mt produced in 2021 (FAOSTAT, 2023). Whilst tomato fruits (and their derivatives) are widely consumed for their flavour, their consumption is also associated with improved health benefits such as reduced incidences of chronic diseases including cancer and cardiovascular disease (Borycka, 2017), resulting in the classification of tomatoes as a "functional food" (Viskelis et al., 2015). Key functional compounds within tomato fruits are carotenoids and phenolic compounds which contribute to a range of properties, including colour (Chaudhary et al., 2018), flavour (Viskelis et al., 2015), and antioxidant activity which is associated with promotion of health (Wise et al., 2020).

Fish processing produces large amounts of waste including heads, skin, and frames (bones), which are high in protein (nitrogen) that can be made plant bioavailable after further processing (hydrolysis) into agricultural products (Madende & Hayes, 2020). Therefore, fish-based agricultural products (emulsified fish waste, *i.e.* 'fish emulsion') have become a popular organic fertiliser N supplement (Ahuja et al, 2020). Fish emulsion (FE) is hypothesised to have biostimulant properties which promote plant growth and vigour via induction of plant endogenous molecular mechanisms including N-metabolism, phytohormone signalling, and defence mechanisms (du Jardin, 2015). Despite the common utilisation of FE during organic crop growth, the biostimulant impacts of FE to functional and hedonic measures of tomato fruits are not well characterised. Accordingly, the aim of this study was to explore the impacts of fertigation supplementation with a biostimulant FE to tomato fruit quality measures.

#### **MATERIALS AND METHODS**

#### *Biostimulant solution*

The biostimulant solution utilised in experiments was a FE produced from fish frames (~42% fish solid, derived from 25 kg/L fish, nutrient profile presented in Table S1, generously provided by Nutrifield Pty Ltd.



## *Tomato growth and sample collection*

Eight 2-week-old seedlings of tomato (*Solanum lycopersicum*) var. Money Maker were planted into eight individual plastic pots (14.5 L Deluxe Pot (300 mm), Garden City Plastics, Dandenong South, VIC), in Coco Perlite substrate (Nutrifield Pty Ltd., Melbourne, VIC) and grown in growth rooms with lighting and environmental conditions as per Wise et al. (2022). For the first 4 weeks the light: dark (L:D) ratio was 18:6 and for the remaining 9 weeks of growth the L:D ratio was 12:12. Each pot contained a 90 cm staked frame for the tomato vines to grow up (4-ring Flower Frame, Jack, Dandenong South, VIC). Seedlings were initially pruned at the apical bud, and four secondary branches were allowed to develop, with all other vegetative buds removed weekly throughout growth. Fertigation was provided to plants daily via a recirculating system wherein each pot contained a single 4 L/h dripper, which delivered the fertigation solution according to the timing schedule (Table 1).

#### **Table 1 : Fertigation delivery schedule**



The fertigation solution consisted of Coco A&B nutrients prepared weekly according to the schedule (Table 2) and corrected to pH 5.8 using pH Up (Nutrifield Pty Ltd., Melbourne, VIC) and pH Down (Nutrifield Pty Ltd., Melbourne, VIC). Four plants received an additional 3 mL/L of the biostimulant solution added into their fertigation solution prior to the pH adjustment. After 13 weeks of growth, ripe fruits were harvested for analyses.

**Table 2 : Fertiliser dose regimen**

Weeks	EC	Coco $A\&B$ concentration (mL/L)			
1	1.1	1.75			
2	1.3	2.25			
$3 - 4$	1.5	2.75			
$5 - 6$	1.8	3.00			
$7 - 8$	2.0	3.50			
$9 - 10$	2.2	4.00			
11	2.0	3.50			
12	1.8	3.00			
13	1.5	2.75			

## *Image analysis*

Tomato fruits  $(n = 3$  per treatment) were imaged and analysed as per Wise et al. (2022) with the following modifications; images were captured against a consistent white background and images were cropped manually (to the edge of the fruit) prior to analyses.

#### *Determination of pH and Brix of crude fruit extract*

Fruit crude extract was produced to measure Brix (ºBx) and pH as per Wise et al. (in press) with the modification of 20-fold dilution with water for pH measurement.

## *Determination of total phenolic and flavonoid content*

Whole fruit dehydration, extraction, quantification of total phenolics via the Folin-Ciocalteu (F-C) method as gallic acid equivalent (GAE), and quantification of flavonoids via the aluminium chloride method as quercetin equivalent (QE) were performed on tomato fruit as per Wise et al. (2023).

## *Sensory perception testing*

A blinded test was conducted to explore if the biostimulant treatment was associated with changes to the sensory perception of fruits. Untrained volunteers  $(n = 9)$  were asked to score the fruits (on a 9-point scale) based on their texture, taste, aroma, mouthfeel, and taste after swallowing (Fig. S1).

## **Statistical analysis**

Statistical analyses of the data were performed in the Minitab 19 statistical software package (Minitab Inc., State College, PA). This involved paired t-tests for sensory analysis data (paired on a per-panellist basis), whilst fruit chemical and colour measures were assessed by ANOVA.

## **RESULTS AND DISCUSSION**

Fruit colour is an important quality metric as it is the consumer's first impression and tomato fruit colour has been identified as a driver of fruit liking (Sinesio et al., 2010). Herein, the impact of FE to tomato fruit colour (Fig. 1, Table S2) was explored, which found that no differences were observed with statistical significance. Of potential note was the intensity of yellow, which was on average 2.16-fold higher in the FE-treatment group than the control group ( $p = 0.120$ ). Therefore, the biostimulant treatment did not cause a significant change in tomato fruit colour.





Fig. 1 : Impact of fish emulsion to fruit colour. Profile of fruit colour, represented as mean image colour proportion  $(n = 3)$ .

The biostimulant treatment produced fruits with a significant 1.25-fold increase to total phenolics  $(p = 0.001)$ , but did not change flavonoids  $(p = 0.418)$ , Fig. 2, Table S3), indicating potential increases to common non-colour-associated tomato phenolics such as the hydroxycinnamic acids; chlorogenic acid, gallic acid, p-coumaric acid, or sinapic acid (Castagna et al., 2014; Perea-Domínguez et al., 2018). It has been recently identified that supplementation of fertiliser with FE during plant growth induced molecular changes associated with pathogen defence responses (Wise et al., 2024) and phenolics are known to be involved in the plant defence response (Kumar et al., 2020). Accordingly, the observed increase to phenolics in tomato grown with FE-supplementation may be indicative of induction of plant defence mechanisms.



Fig. 2 : Fish emulsion impact to tomato antioxidants. A) total phenolic, and B) flavonoids of tomato fruit. Data presented as mean  $\pm$  standard deviation (n = 3), with significant differences indicated by \*\*\* for  $p< 0.001$ .

**Table 3 : Impact of fish emulsion to tomato chemical measures**

Measure	Control		<b>Fish emulsion</b>		P-value
	Mean	SD	Mean	SD	
Brix $(°)$	4.367	0.306	4.000	0.100	0.187
pH (20-fold diluted)	4.167	0.289	3.833	0.289	0.230





Differences in Brix between control and FE-treated fruit extracts were not statistically significant  $(p = 0.119,$  Table 3), which is consistent with the nonsignificant differences in sweet taste ( $p = 0.403$ , Table 4), as Brix (or "total soluble solids") strongly correlate with fruit sweet perception (Harker et al., 2002; Malundo et al., 2001). Similarly, differences in pH between control and FE-treated fruit extracts were not statistically significant ( $p = 0.230$ , Table 3), which is consistent with non-significant differences in sour taste  $(p = 0.081,$  Table 4), as pH strongly correlates with fruit sour perception (Malundo et al., 2001).

Within tomatoes, the phytochemicals associated with fruit colour are the carotenoids; lycopene, β-carotene and lutein. Lycopene is the primary contributor to red colour of tomatoes (Kotikova et al., 2009), β-carotene contributes to orange colour, and xanthophylls (such as lutein) contribute to yellow colour (Becerra et al., 2020; Giuliano et al., 1993). Accordingly, the unchanged levels of red and orange colour (Fig. 1) indicate no significant changes to lycopene and betacarotene, but the on-average 2.16-fold increase to yellow ( $p = 0.120$ ) suggests that FE-treatment may be associated with promotion of lutein production. Although lutein is relatively lipophilic (Li et al., 2020) and the F-C assay has a tendency not to capture contributions of lipophilic antioxidants (Berker et al., 2013), lutein is known to contribute to antioxidant activity (Krinsky et al., 2003), and Everette et al. (2010) showed that compounds related to lutein demonstrate activity for the F-C assay. Accordingly, it is possible that lutein has contributed to the apparent increase in phenolics via antioxidant activity. Nonetheless, the increase in measured phenolics, either as a genuine increase in phenolics, or an increase to antioxidant-active phytochemicals (such as carotenoids), suggests that the FE-treatment has enhanced the functional food value of tomato fruit. Thus, inclusion of FE during tomato cultivation may produce fruit with enhanced antioxidant potential which is associated with improved potential to promote health via mediation of diseases associated with oxidative stress and inflammation (Bungau et al., 2019).

## **CONCLUSION**

Benefits to crop production from application of FE has tended to focus on nutritional supplementation, however, recent publications indicate that this complex functions as a biostimulant to promote yield quality. The results herein indicate that supplementation of hydroponic nutrients with FE during the growth of tomatoes resulted in increased antioxidant potential (attributed to phenolics and potentially also carotenoids), thereby increasing functional food value without significantly impacting customer perceptions of fruit quality or desirability. Accordingly, FE appears to be a valuable opportunity for tomato farmers to increase the functional food value of their crops and thereby provide increased health benefits to their customers.

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