

Review article

The fatty acid composition of black soldier fly larvae: the influence of feed substrate and applications in the feed industry

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Supplementary material

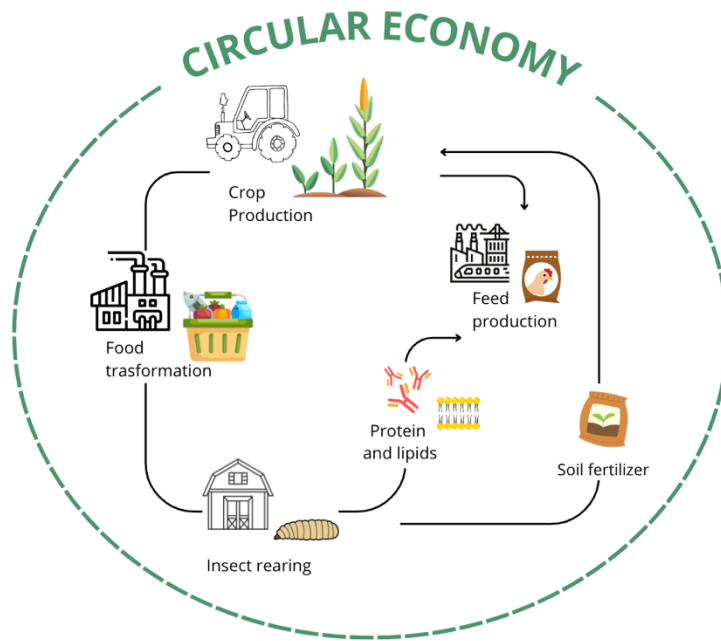


FIGURE S1 A model of circular economy achieved throughout the bioconversion of BSF larvae. Starting with organic substrates from crop production and/or food transformation, insect proteins and lipids can be generated for use in the feed sector, as well as insect fertilizer suitable for crop production.







Permitted substrates for insect rearing	Prohibited substrates for insect rearing
Reg. EC 767/2009, EC 1069/2009, EU 142/2011	Reg. EC 767/2009, EC 1069/2009, EU 142/2011
 Plant-Based Biomass	 Meat and derivatives
 Eggs	 Solid/food waste
 Milk and derivatives	 Manure

FIGURE S2 Graphical representation of the permitted and prohibited feed substrates for insects to be used in animal feeds.

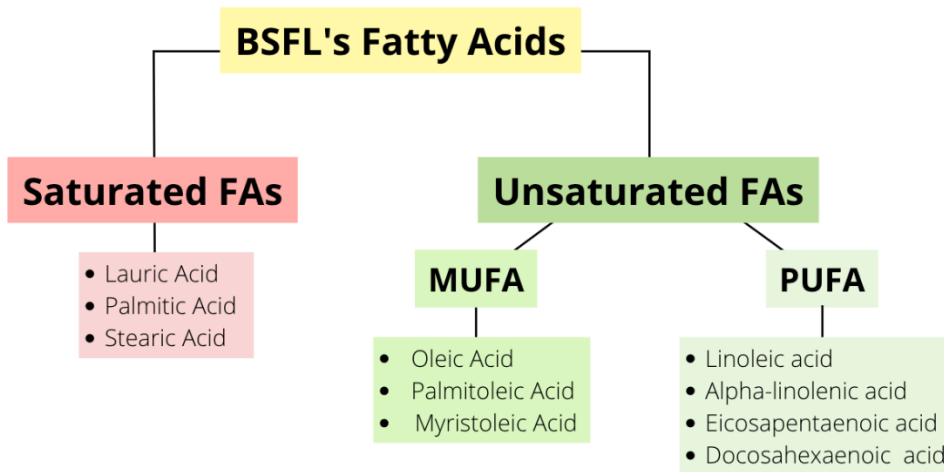


FIGURE S3 Fatty acid content of BSF larvae: their groups and main components.

TABLE S1 Literature analyzing the effects of rearing substrate on BSF fatty acid composition

Reference	Substrate tested and reference numbers (used in Table S2)
Fischer and Romano (2021)	<ol style="list-style-type: none"> 1. 50% coffee grounds + 50% donut dough 2. 100% coffee grounds 3. 100% donut dough
Galassi <i>et al.</i> (2021)	<ol style="list-style-type: none"> 4. Okara 5. Maize distillers 6. Brewery by-products
Romano <i>et al.</i> (2021)	<ol style="list-style-type: none"> 7. Sweet potato 8. Spent coffee 9. Dough
Schreven <i>et al.</i> (2021)	<ol style="list-style-type: none"> 10. Chicken feed substituted (dry matter basis) with 0, 25, 50, or 100% crambe press cake 11. Chicken feed substituted (dry matter basis) with 0, 25, 50, or 100% crambe seed meal 12. Chicken feed substituted (dry matter basis) with 0, 25, 50 or 100% camelina press cake

	13. Chicken feed substituted (dry matter basis) with 0, 25, 50 or 100% camelina seed meal
Campbell <i>et al.</i> (2020)	14. Brewery by-products
Ewald <i>et al.</i> (2020)	15. Fresh mussels 16. Ensiled mussels 17. Rotten mussels 18. Food waste 19. Rainbow trout (<i>Onchorhynchus mykiss</i>) 20. Bread
Kierończyk <i>et al.</i> (2020)	21. Wheat bran 22. Carrots 23. Cabbage 24. Potatoes 25. Wheat bran, carrots, cabbage, potatoes 1:1:1:1
Oonincx <i>et al.</i> (2020)	26. Chicken feed 100%

	27. Chicken feed enriched with 1, 2, or 4% flaxseed oil
Riudavets <i>et al.</i> (2020)	28. Apricots 29. Brewer's spent grains and malt bagasse 30. Brewer's spent yeast (from a brewery) 31. Feed mill by-products 32. Hatchery waste
Truzzi <i>et al.</i> (2020)	33. Coffee silverskins enriched with 5, 10, 20 or 25% Schyzochytrium sp. 34. Coffee silverskins enriched with 5, 10, 20 or 25% Isochrysis sp.
Barroso <i>et al.</i> (2019)	35. Discarded round sardinella (<i>Sardinella aurita</i>)
Cullere <i>et al.</i> (2019)	36. 50% layer mash + 50% fish offal
Meneguz <i>et al.</i> (2018)	37. Fruit (apples 47.8%, oranges 15.5%, apple leftovers 13.8%, strawberries 7.1%, mandarin oranges 4.8%, pears 4.1%, kiwis 3.4%, bananas 1.9% and lemons 1.6%) 38. Fruit and vegetable (celery 43.4%, oranges 28.9% and peppers 27.7%) 39. Brewery by-products

	40. Winery by-products
Moula <i>et al.</i> (2018)	41. 100% horse manure
Jucker <i>et al.</i> (2017)	42. FRU: apple (<i>Malus domestica</i> Borkh. var. golden delicious), pear (<i>Pyrus communis</i> L. var. Kaiser), orange (<i>Citrus sinensis</i> (L.) 43. VEG: lettuce (<i>Lactuca sativa</i> L.), string green beans (<i>Phaseolus vulgaris</i> L.), and cabbage (<i>Brassica oleracea</i> var. capitata.) 44. MIX: 50% FRU + 50% VEG
Liland <i>et al.</i> (2017).	45. Brown algae (from 10 to 100%, together with control diet)
Oonincx <i>et al.</i> (2015)	46. HPHF (high protein, high fat): spent grains 60%, beer yeast 20%, cookie remains 20% 47. HPLF (high protein, low fat): beer yeast 50%, potato steam peelings 30%, beet molasses 20% 48. LPHF (low protein, high fat): cookie remains 50%, bread 50% 49. LPLF (low protein, low fat): steamed potato peelings 30%, beet molasses 20%, bread 50%
Makkar <i>et al.</i> (2014)	50. Cow manure 51. 50% cow manure + 50% fish offal

	52. Swine manure
St-Hilaire <i>et al.</i> (2007b)	53. Cow manure 100%, 54. Cow manure 50% + fish offal 50% 55. Fish offal 25% + cow manure 75% 56. Fish offal 10% + cow manure 90%

TABLE S2 (1), (2), (3) Fatty acid profile of BSF reared on different diets (see Table S1), expressed as a percentage of total fatty acids, (mean \pm standard deviation), unless otherwise specified

(1)

<i>Diet</i>	Fruit and vegetable	Fruit and Vegetable	Fruit	Vegetable	Brewer's by-products	Brewer's by-products	Brewer's by-products	Winery by-products	HPHF	HPLF	LPHF	LPLF
	(% of total FA)	(g/100g total fatty acids).	(g/100g total fatty acids)	(g 100 g ⁻¹ FA)	(g/100 g total fatty acids)	(g/100g total fatty acids)		(g/100g total fatty acids)				
<i>Reference App. B</i>	44.0	38.0	37.0	25.0	14.0	39.0	6.0	40.0	46.0	47.0	48.0	49.0
<u>SFA</u>	65.0 \pm 0.6	78.9 \pm 1.1	81.9 \pm 0.5	68.6	64.5	61.2 \pm 0.8		63.0 \pm 1.7				

<u>MUFA</u>	11.0 ± 0.1	912.3 ± 0.7	13.3 ± 0.3	14.0	12.3	12.7 ± 0.5		18.9 ± 0.6				
<u>PUFA</u>	24.1 ± 0.3	8.8 ± 0.7	4.8 ± 0.2	7.7	19.6	26.0 ± 0.7		18.0 ± 1.5				
C10:0	1.0±0.1			1.3	0.92				0.7 ± 0.1	1.3 ± 0.1	0.8 ± 0.1	1.2 ± 0.0
C12:0	41.5±0.6	52.1±1.8	57.43±1.10	42.1	36.0	32.4±0.9	28.9	34.7±1.7	28.9 ± 1.0	48.4 ± 1.5	38.4 ± 6.5	50.7 ± 4.2
C12:1												
C14:0	7.4±0.1	10.3±0.3	9.6±0.3	8.2	7.83	6.6±0.3	5.83	6.5±0.4	7.4 ± 0.1	9.5 ± 0.4	7.8 ± 0.4	9.0 ± 0.1
C14:1					0.15				0.4 ± 0.0	0.9 ± 0.1	0.6 ± 0.0	0.7 ± 0.0
C15:0					0.21							
C15:1					0.10							
C16:0	12.8±0.3	13.9±0.7	13.0±0.46	13.2	16.8	20.4±0.6	20.5	18.9±0.7	17.0 ± 0.1	11.8 ± 0.8	14.4 ± 1.7	11.6 ± 1.2
C16:1	1.4±0.1(n-7)	3.3±0.4 (c9)	3.7±0.1	4.3	2.29	2.9±0.3	4.26	6.1±0.5	2.9 ± 0.1	6.6 ± 0.9	3.4 ± 0.1	4.7 ± 0.5
C17:0									0.5 ± 0.1	0.1 ± 0.0	0.6 ± 0.2	0.2 ± 0.0
C17:1												
C18:0	1.7±0.1	2.6±0.2	1.7±0.1	2.7	2.28	1.8±0.1	3.10	2.8± 0.2	2.8±0.1	2.0 ± 0.0	2.4 ± 1.0	1.8 ± 0.4
C18:1 Δ 9	8.7±0.2	8.53±0.40	9.3±0,2	8.3	9.38	9.2±0.2	11.2	12.4±0.4	15.9±0.3	10.8±1.1	18.1±1.8	10.3 ± 0.9

C18:1 Δ 11		0.4±0.0	0.3±0.0	0.5		0.6±0.1		0.4±0.0	0.3 ± 0.0	0.5 ± 0.1	0.2 ± 0.0	0.2 ± 0.1
C 18:2 n-6	21.2±0.4	7.0±0.7	4.1±0.2		18.1	3.5±0.6	21.4	17.6±1.5	17.1 ± 0.3	3.6 ± 1.6	8.3 ± 0.8	6.0 ± 0.6
C 18:2 n-3					1.48							
C18:3 n-3	2.6±0.1	1.73±0.1	0.7±0.1			2.4±0.1	1.92	0.4±0.0	1.5 ± 0.0	0.6 ± 0.2	0.8 ± 0.0	1.0 ± 0.1
C20:0				<0.1	0.11							
C20:1 n-9				<0.1	0.19							
C20:2												
C20:3												
C20:4 n-6									0.2 ± 0.0	0.6 ± 0.16	0.1 ± 0.0	0.1 ± 0.0
C20:5 n-3												
C21												
C22:0												
C22:1												
C22:6 n-3												

HPHF = high protein, high fat; HPLF = high protein, low fat; LPHF = low protein, high fat; LPLF = low protein, low fat.

(2)

<i>Diet</i>	Sweet potato	Maize Distiller	Okara	Donut dough	Dough	Bread	Coffee silverskins + algae	Coffee silverskins + algae	Spent coffee	Spent coffee	Donut + spent coffee	Fresh mussels	Ensiled mussels
	(% of FA)			(mg/g “as is basis”)	(% FA)	(% of the identified FA expressed on DM)	(g/100g FAs)	(g/100g FAs)	(mg/g “as is basis”)	(% FA)	(mg/g “as is basis”)	(% of the identified FA expressed on DM)	(% of the identified FA expressed on DM)
<i>Reference App. B</i>	7	5	4	3	9	20	33	34	2	8	1	15	16
<u>SFA</u>						75.6 ± 1.4						73.7 ± 1.6	45.3 ± 3.5
<u>MUFA</u>						15.1 ± 1.4						19.5 ± 1.4	32.1 ± 0.4
<u>PUFA</u>						9.3 ± 0.2						6.8 ± 0.3	22.06 ± 3.1
C10:0				4.59 ± 0.2			0.25±0.0	0.85±0.0	2.19 ± 0.2		3.82 ± 0.2		
C12:0	42.9 ± 4.2	31.6	17.6	158.8±9.7	40.7±1.2	51.8±3.3	14.1±1.5	19.5±0.3	66.4 ± 6.4	27.5 ± 3.6	158.9 ± 6.52	52.1±1.8	13.4±3.9
C12:1													
C14:0	7.5 ± 0.7	6.6	3.3	19.8±1.2	6.7±0.6	9.5±0.9	3.2±0.7	5.9±0.9	11.4 ± 0.9	5.0 ± 0.4	24.4 ± 1.1	8.0±0.5	5.8±0.4

C14:1	0.2±0.0 (n-9)			0.2±0.0 (n-5)	0.3±0.0 (n-9)	0.2±0.0 (n-5)			0.45±0.0 (n-5)	0.1 ± 0.0 (n-9)	0.6 ± 0.1 (n-5)	0.3±0.0 (n-5)	0.4±0.1 (n-5)
C15:0	0.2±0.0			0.3±0.0	0.1±0.0		0.5±0.0	0.2±0.0	0.2 ± 0.0	0.2 ± 0.0	0.6 ± 0.0		
C15:1				0.0±0.0 (n-5)					0.0 ± 0.0 (n5)		0.0 ± 0.0 1.0 (n-5)		
C16:0	17.5 ± 1.3	13.6	14.0	30.8±2.0	15.2±0.9	12.7±0.8	18.1±2.0	12.1±0.6	62.9 ± 3.9	24.9 ± 1.9	38.3 ± 3.3	11.9±1.3	21.9±0.6
C16:1		1.1	1.54	1.1±0.1 (n-7)		2.8±0.1 (n-7)	4.7±0.5 (n-7)	4.3±0.5 (n7)	9.1 ± 0.5 (n-7)		9.3 ± 0.3 (n7)	6.9±0.8 (n7)	14.1±0.4 (n-7)
C17:0				0.4±0.0			0.5±0.0	0.2±0.0	0.6 ± 0.0		0.6 ± 0.0		
C17:1 n10													

C18:0	2.6±0.1	2.1	3.3	1.5±0.2	1.9±0.1	1.5±0.3	10.8±0.7	4.7±0.6	7.7 ± 0.2	2.9 ± 0.1	5.2 ± 0.5	1.6±0.3	4.0±0.3
C18:1 Δ 9	10.4±0.6	15.9	23.5	25.1 ± 2.2 (n-9)	9.7±0.6	12.0±1.2	9.0±0.9	11.7±0.8	26.6 ± 1.2	10.5 ± 0.2	27.2 ± 2.0	10.3±2.3	14.0±0.4
C18:1 Δ 11				25.1 ± 2.2									
C 18:2n-6	12.4±2.5	24.3	28.4		18.9±1.1	7.7±0.3	6.2±0.6	37.9±0.2		23.3 ± 1.5		2.6±0.3	4.5±0.4
C 18:2n-3			2.85	52.9±6.1						2.0 ± 0.2			
C18:3 n-3	1.6±0.4	0.7			0.7±0.1	1.6±0.1	1.0±0.2	1.0±0.1				1.3±0.2	3.6±0.6
C20:0	0.1±0.01			5.9±0.2	0.1±0.0		11.0±0.3	1.2±0.1	0.6 ± 0.0	0.2 ± 0.0	0.2 ± 0.0		

C20:1 n-9	0.6±0.1			0.0 ±0.0	9.7±0.6		0.0±0.0	0.0±0.0	0.3 ± 0.0	0.6 ± 0.3	0.1 ± 0.0		
C20:2													
C20:3													
C20:4 n-6	0.1±0.0			0.0±0.0	0.0±0.0	0.0±0.0	0.1±0.3	3.6±0.3		0.1 ± 0.0		0.2±0.0	1.3±0.2
C20:5 n-3	0.2±0.1			0.0±0.0	0.0±0.0	0.0±0.0	0.8±0.2	10.6±0.2	0.1 ± 0.0	0.2 ± 0.0	0.0 ± 0.0	2.0±0.2	8.2±1.0
C21									0.1 ± 0.0		0.4± 0.1		
C22:0				0.0±0.0			16.0±0.4	1.3±0.2	0.1 ± 0.0 (n-9)		0.1 ± 0.0 (n-9)		
C22:1				0.0±0.0 (n-9)					0.0 ± 0.0 (n-3)		0.0 ± 0.0 (n-3)		
C22:6 n-3	0.0±0.0			0.0±0.0		0.0±0.0	0.7±0.2	16.7±0.3	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5± 0.0	4.5±0.9

(3)

Diet	Rotten mussels	Fish	Cow manure	Cow manure + fish offal	Swine manure	Horse manure	Food waste	Layer mash + fish offal	Chicken feed	Chicken feed + 4% flaxseed	Brown algae (10%)
	(% of the identified FA expressed on DM)	(% of the identified FA expressed on DM)	(% fatty acids)	(% fatty acids)	(% fatty acids)	(% of TFA)	(% of the identified FA expressed on DM)	(% of total FAMES)	(% TFA)	(% TFA)	(area %)
<i>□ Reference App. B □</i>	<i>□17□</i>	<i>□19□</i>	<i>□50□</i>	<i>□51□</i>	<i>□52□</i>	<i>□41□</i>	<i>□85□</i>	<i>□36□</i>	<i>□26□</i>	<i>□27□</i>	<i>□45□</i>
<u>SFA</u>	65.6 ± 4.7	49.6 ± 1.6					65.2 ± 3.7	72.0	74.4 ± 1.0	63.5 ± 2.8	67.2
<u>MUFA</u>	26.2 ± 3.7	31.8 ± 1.2					22.4 ± 2.6	18.7	15.1 ± 0.5	15.6 ± 1.2	13.2
<u>PUFA</u>	8.2 ± 1.0	18.7 ± 0.4					12.4 ± 1.1	12.6	10.1 ± 0.7	20.3 ± 1.6	19.2
C10:0						2.8		1.02	1.1±0.0	0.9 ± 0.1	
C12:0	32.3±7.6	28.6±2.1	21.4	49.3	42.6	28.1	39.9±5.1	48.3	47.8±1.2	43.2 ± 3.3	37.9

C12:1											
C14:0	10.1±0.1	6.1±0.4	2.9	6.8	6.9	6.7	6.7±0.3	6.8	9.2±0.2	8.4 ± 0.5	9.4
C14:1	0.6±0.0 (n-5)	0.2±0.0 (n-5)					0.2±0.0 (n-5)	0.20			
C15:0								0.03			
C15:1								0.0			
C16:0	19.8±2.1	12.6±0.4	16.1	10.5	11.1	22.0	16.3±1.0	13.4	13.7±0.3	12.8 ± 0.5	16.6
C16:1	9.8±1.2 (n-7)	4.8±0.5 (n-7)		3.5 (n-7)		8.1 (n-7)	2.6±0.3 (n-7)	3.8	2.5 ± 0.12	1.9 ± 0.1	1.8 (n-7)
C17:0						0.8		0.1			
C17:1 n10								0.1			
C18:0	3.3±0.6	2.2±0.2	5.7	2.78	1.3	5.1	2.1±0.6	2.1	2.3±0.1	2.6 ± 0.3	3.2

C18:1 Δ 9	12.9±2.1	25.1±0.7	32.1	11.8	12.3	22.9	19.1±2.1	13.3	11.7±0.6	12.5 ± 0.9	9.1
C18:1 Δ 11								1.0			
C 18:2n-6	4.2±0.7	12.5±0.3	4.5	3.7	3.6	2.1	9.9±0.8	4.3	9.1±0.8	10.0 ± 1.2	15.1
C 18:2n-3											1.2
C18:3 n-3	1.1±0.1	3.4±0.1	0.19	0.08	0.74	<0.5%	1.8±0.2	0.2	0.5±0.1	5.5 ± 0.6	
C20:0								0.0			
C20:1 n-9								0.1			
C20:2								0.6 (n-6)			
C20:3								0.1 (n-3)			
C20:4 n-6	0.1±0.0	0.1±0.0					0.2±0.0	0.1			0.4
C20:5 n-3	1.9±0.2	1.7±0.1	0.03	0	1.66	<0.5%	0.5±0.0	1.0			0.2
C21											

C22:0								0.0			
C22:1											
C22:6 n-3	0.3±0.0	0.7±0.0	0.006	0	0,59	<0.5%	0.0 ±0.0				




Animal species' supplemented/integrated with BSF fat	Effects
<p data-bbox="496 510 651 544"><u>Acquaculture</u></p> 	<ul data-bbox="794 427 1171 607" style="list-style-type: none"> • No negative effects on growth performances and feed utilization • positive effect on immune system activity and modulation of gut microbiota • Higher C14:0, C16:0, SFA in muscle and liver of the animals
<p data-bbox="533 734 619 768"><u>Poultry</u></p> 	<ul data-bbox="794 651 1145 853" style="list-style-type: none"> • No negative effect on growth performances • No difference in sensory and quality characteristics of the final products • Positive effects on gut bacterial population • Anti-inflammatory properties • Improved FCR
<p data-bbox="549 898 603 931"><u>Pigs</u></p> 	<ul data-bbox="794 898 1082 931" style="list-style-type: none"> • Improved efficiency and ADG

FIGURE S4 Positive effects of BSF larvae fat supplementation for the feed sectors, aquaculture, poultry and pigs respectively.

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