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Fatty acid profile of black soldier fly larvae reared on winery by-products

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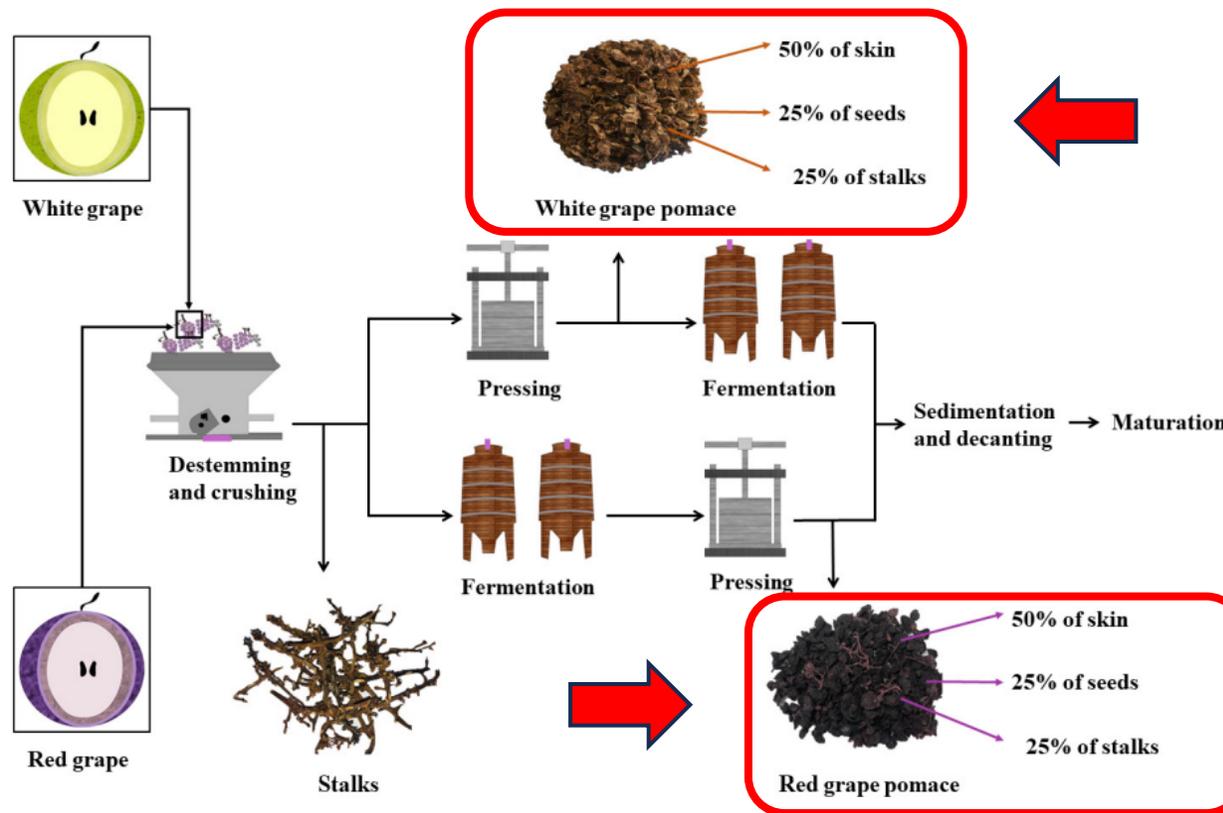
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INTRODUCTION

Grapes



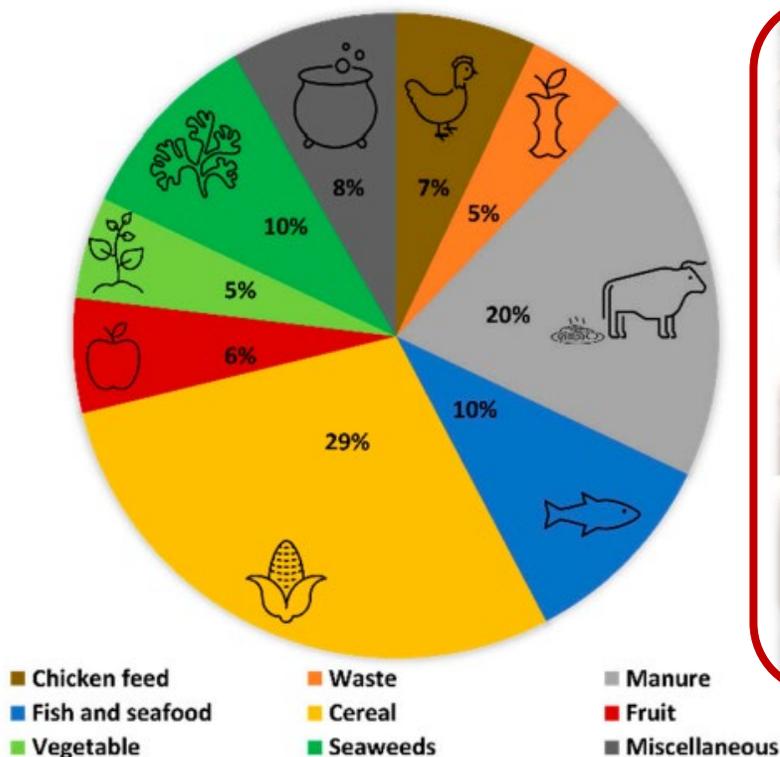
Global surface area: 7.3×10^6 ha (OIV, 2023)

Grape production: 8.0×10^7 tonnes (OIV, 2023)

↳ in Italy 7.5×10^6 tonnes (ISTAT, 2024)

Hoss et al. (2021)

INTRODUCTION



AIM



to evaluate the effects of **grape pomace** from **two different varieties** as rearing substrate for **black soldier fly larvae**, with a particular focus on the impact on their **fatty acid profile**

Top 10 substrates used to feed *Hermetia illucens* larvae according to publications retrieved from Web of Science and Scopus concerning *H. illucens* larvae and their fatty acid profile (Rodrigues et al., 2022)



MATERIALS & METHODS

Experimental design

2 grape varieties



Becuet

6 kg skins + 1.25 kg seeds



Moscato

6 kg skins + 1.25 kg seeds



- 12 containers ($23 \times 30 \times 9 \text{ cm}^3$) - 6 replicates / rearing substrate
- 1220 g (40% of DM) of rearing substrates
- 1000 6-day old larvae in each container
- at the end of the larval growth: 30 larvae / replica collected for FA analysis

Image by Bellezza Oddon et al. (2024)

MATERIALS & METHODS

Laboratory analyses



grape pomace



- DM, ash, CP, EADF, ADL (AOAC methods, 2000; 2003)
- NDF (Mertens, 2002)
- Total lipids (Rodriguez-Estrada et al., 1997)
- FA composition (Alves et al., 2008; Renna et al., 2014)
- Phenolic compounds: TEP, TT, NTP, CT and HT (Iussig et al., 2015)

black soldier fly larvae



- Total lipids (Rodriguez-Estrada et al., 1997)
- FA composition (Kramer et al., 1997; Mele et al., 2014)
- CLA isomeric profile (Conte et al., 2019)



Statistical analysis



Independent-sample Student's t-tests; significance declared at $P \leq 0.05$.



RESULTS & DISCUSSION

Proximate composition (g/100 g DM) and phenolic fractions (g/kg DM) of grape pomace



	Becuet	Moscato
Dry matter (g/100 g)	94.06	88.18
Ash	13.27	5.83
Crude protein	11.75	10.11
Neutral detergent fibre	49.48	37.96
Acid detergent fibre	34.48	34.18
Acid detergent lignin	22.77	19.99
Non-structural carbohydrates	19.17	40.19



	Becuet	Moscato
Total extractable phenols	173.7	156.9
Non-tannin phenols	5.0	4.0
Total tannins	168.7	152.9
Condensed tannins	5.3	19.3
Hydrolysisable tannins	163.4	133.6

RESULTS & DISCUSSION

Total lipids (g/100 g wet basis) and fatty acid profile (g/100 g TL) of grape pomace

	Becuet	Moscato
Total lipids	5.96	5.22
C10:0	0.14	0.10
C12:0	0.17	0.07
C14:0	0.22	0.11
C15:0	0.05	0.05
C16:0	9.22	9.63
C18:0	4.01	2.81
C20:0	0.62	1.00
Σ SFA	14.43	13.77



	Becuet	Moscato
C16:1 <i>c9</i>	0.31	0.32
C18:1 <i>t9-11</i>	0.04	0.07
C18:1 <i>c9</i>	13.66	11.47
C18:1 <i>c11</i>	0.76	1.09
C20:1 <i>c11</i>	0.18	0.16
Σ MUFA	14.95	13.11

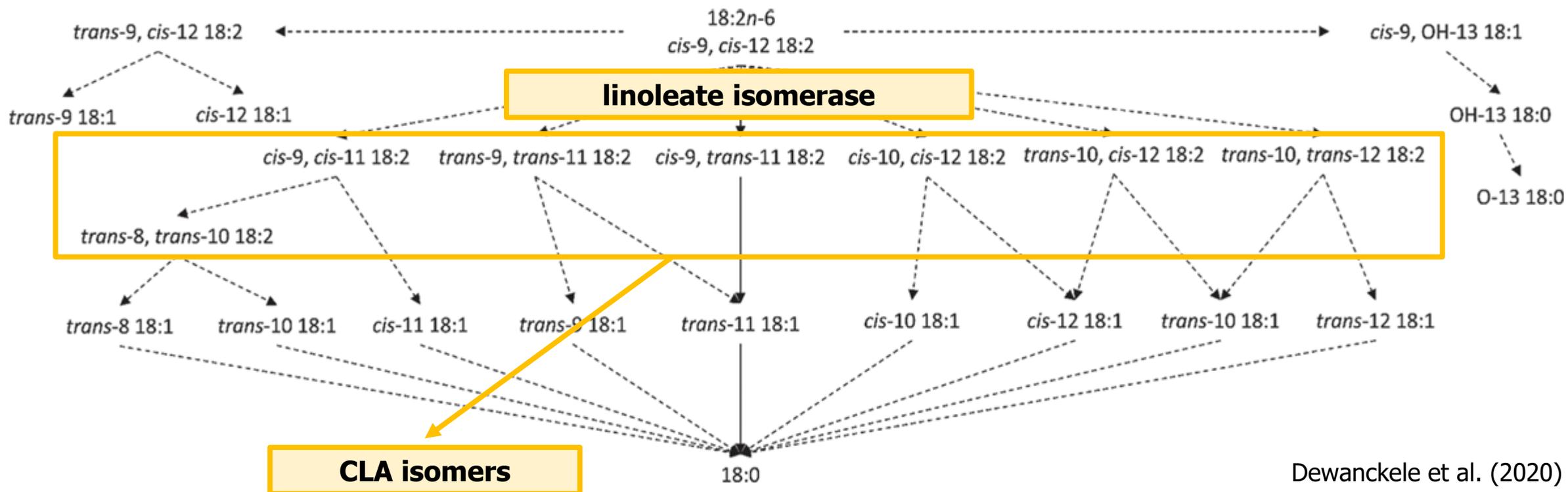
	Becuet	Moscato
C18:2 <i>c9c12</i>	59.15	53.16
C18:2 <i>c9t11</i>*	0.04	0.10
C18:2 <i>t9t11</i>	0.05	0.06
C18:3 <i>c9c12c15</i>	1.39	2.31
Σ PUFA	60.54	55.47
Σ CLA	0.09	0.16

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; CLA, conjugated linoleic acid; * coeluted with C18:2 *t7c9* and C18:2 *t8c10*.

RESULTS & DISCUSSION

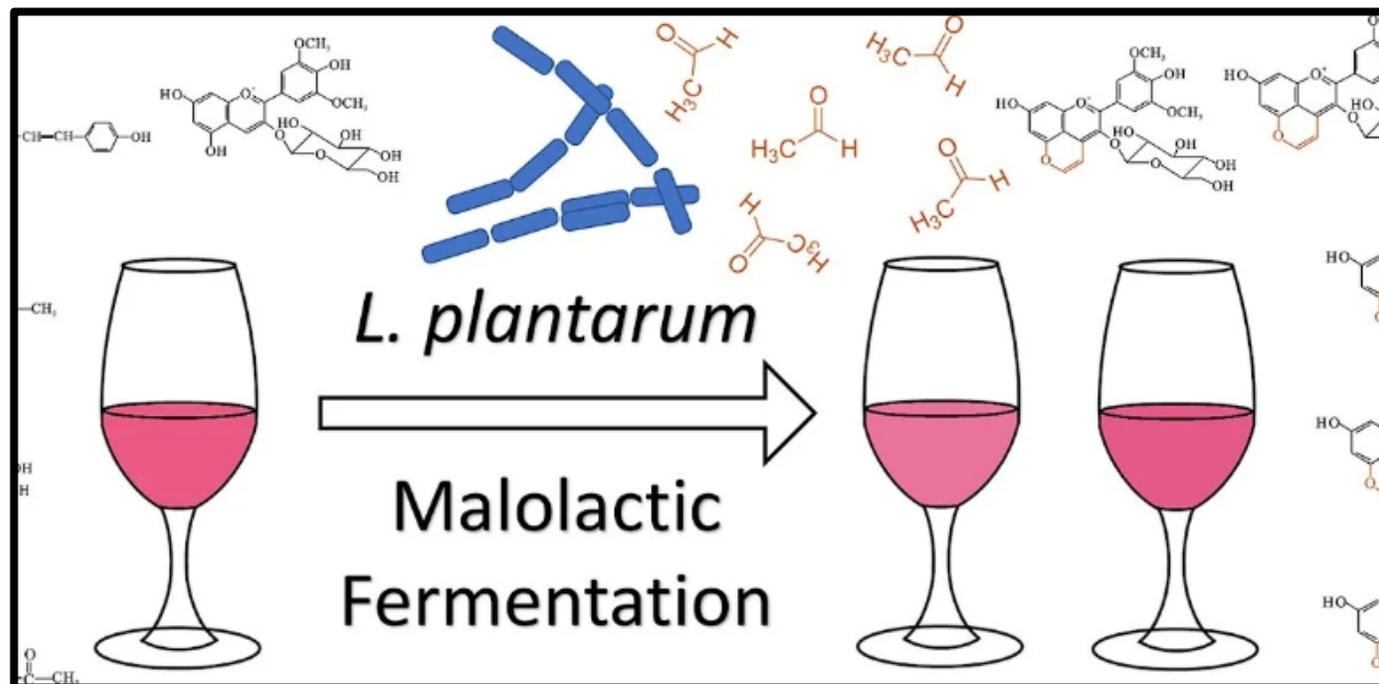
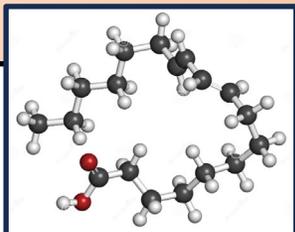
The presence of conjugated linoleic acid (CLA) in grape pomace

Biohydrogenation of C18:2 n-6 inside the rumen



RESULTS & DISCUSSION

The presence of conjugated linoleic acid (CLA) in grape pomace



Lactobacillus plantarum

- malolactic starter inoculated during the winemaking process
- one of the most efficient CLA producers

Image: www.reddit.com

RESULTS & DISCUSSION



Straight-chain saturated fatty acids in black soldier fly larvae (BSFL)

	B-fed BSFL	M-fed BSFL	SED	P
C10:0	0.33	0.50	0.043	0.003
C12:0	12.94	18.05	1.185	0.002
C14:0	2.17	3.63	0.168	0.000
C16:0	9.01	11:44	0.512	0.003
C18:0	2.03	2.69	0.197	0.057
C20:0	0.11	0.11	0.024	0.963
C22:0	0.08	0.07	0.021	0.795
C23:0	0.16	0.03	0.040	0.008
C24:0	<0.001	0.01	0.009	0.197
Σ Straight-chain SFA	27.59	37.09	1.589	0.000

- various insect species are able to biosynthesise *de novo* individual SFA from C12 to C18 using dietary carbohydrates
- acetyl-CoA carboxylase and fatty acid synthase have been characterised in black soldier fly
- straight-chain SFA mainly biosynthesised by black soldier fly larvae starting from non-structural rather than structural carbohydrates**



further trials needed

RESULTS & DISCUSSION



Odd- and branched-chain fatty acids in black soldier fly larvae

- **odd-chain fatty acids** (C15:0 and C17:0) **may be of microbial origin** and/or may derive from α -oxidation of even-chain fatty acids (C16:0 and C18:0, respectively) ?

- BCFA a result of the **presence of *Lactobacillus spp.* in the gut** of the larvae ?

- a higher presence of leucine, isoleucine, and valine in the gut of larvae fed Becuet, up- or down-regulation of enzymes involved in BCFA synthesis, or greater accumulation of *Lactobacillus spp.* in the gut of the larvae from the rearing medium ?

further trials needed

	B-fed BSFL	M-fed BSFL	SED	P
C15:0	0.23	0.23	0.027	0.890
C17:0	0.29	0.34	0.042	0.311
C15:0 iso	0.20	0.14	0.037	0.175
C15:0 anteiso	0.29	0.06	0.024	0.000
C16:0 iso	0.14	0.08	0.023	0.044
C17:0 iso	0.59	0.52	0.029	0.030
C18:0 iso	0.86	0.60	0.088	0.015
Σ Branched-chain FA	2.07	1.40	0.129	0.000



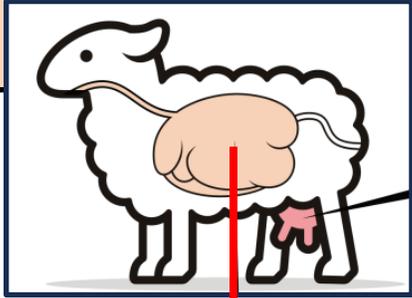
RESULTS & DISCUSSION



Monounsaturated fatty acids in black soldier fly larvae

	B-fed BSFL	M-fed BSFL	SED	P
C16:1 t9	0.15	0.01	0.027	0.001
C16:1 c9	0.99	1.28	0.099	0.013
C17:1 c9	0.11	0.12	0.022	0.635
C18:1 t9	0.01	<0.001	0.007	0.106
C18:1 t10	n.d.	n.d.	-	-
C18:1 t11	0.21	0.01	0.086	0.069
C18:1 c9	11.41	14.03	0.603	0.001
C18:1 c11	0.53	0.54	0.047	0.778
C18:1 c13	0.31	<0.001	0.013	0.000
Σ MUFA	14.04	16:28	0.739	0.013

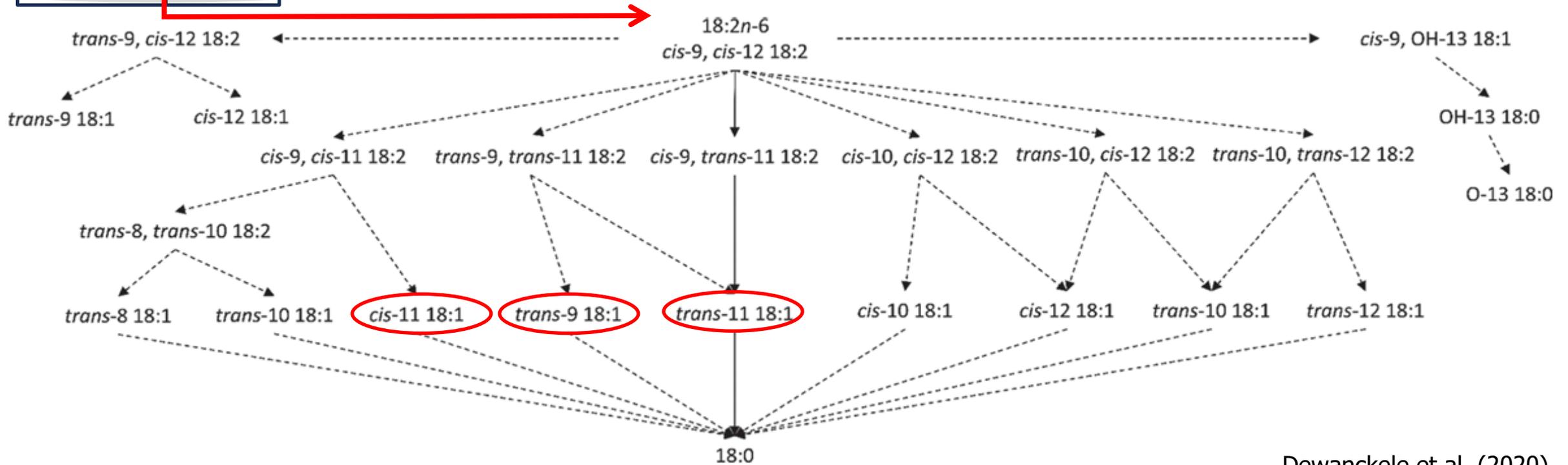
- **oleic acid is both synthesized *de novo* by the larvae and bioaccumulated from the rearing substrate**
- **Δ9-desaturase in black soldier fly larvae, similarly to other Diptera**



RESULTS & DISCUSSION



Monounsaturated fatty acids in black soldier fly larvae



Dewanckele et al. (2020)

RESULTS & DISCUSSION



Polyunsaturated fatty acids in black soldier fly larvae

	B-fed BSFL	M-fed BSFL	SED	P
C18:2 n-6	30.66	24.75	1.512	0.003
C18:3 n-3	0.40	0.57	0.032	0.000
C18:4 n-3	0.14	0.02	0.028	0.002
C20:2 n-6	0.14	0.25	0.050	0.044
C20:3 n-6	0.06	<0.01	0.031	0.166
C20:4 n-6	0.17	0.04	0.039	0.018
C20:5 n-3	0.21	0.31	0.038	0.019
C22:3 n-3	0.15	0.19	0.026	0.166
Σ PUFA	34.87	26.25	1.313	0.000
PUFA n-6 / PUFA n-3	35.06	23.31	2.787	0.002

- higher C18:2 n-6 in B-fed larvae reflects differences in the grape pomace varieties
- part of C18:2 n-6 may derive from **Δ12-desaturase activity on C18:1 n-7**





RESULTS & DISCUSSION



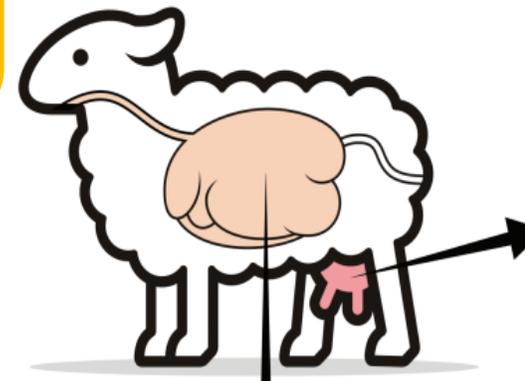
Conjugated linoleic acid in black soldier fly larvae

	B-fed BSFL	M-fed BSFL	SED	P
C18:2 c9t11	2.74	0.02	0.691	0.011
C18:2 t7c9	0.17	0.02	0.036	0.005
C18:2 t10c12	0.03	0.01	0.006	0.007
Σ CLA	2.95	0.05	0.727	0.010

- both CLA c9t11 and t7c9 may have been bioaccumulated in the larvae starting from the grape pomace

- Δ9-desaturase in the larvae → CLA c9t11 could have been de novo biosynthesized using C18:1 t11 as precursor

but CLA t7c9?



Δ9-desaturase

C18:1 t11 → C18:2 c9t11

C18:1 t7 → C18:2 t7c9



RESULTS & DISCUSSION



Conjugated linoleic acid in black soldier fly larvae

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- greater linoleic acid content in Becuet pomace → high linoleate isomerase activity found in numerous *Lactobacillus* strains → *Lactobacillus* spp. in the gut of black soldier fly larvae → > CLA c9t11 and t10c12 in Becuet-fed larvae

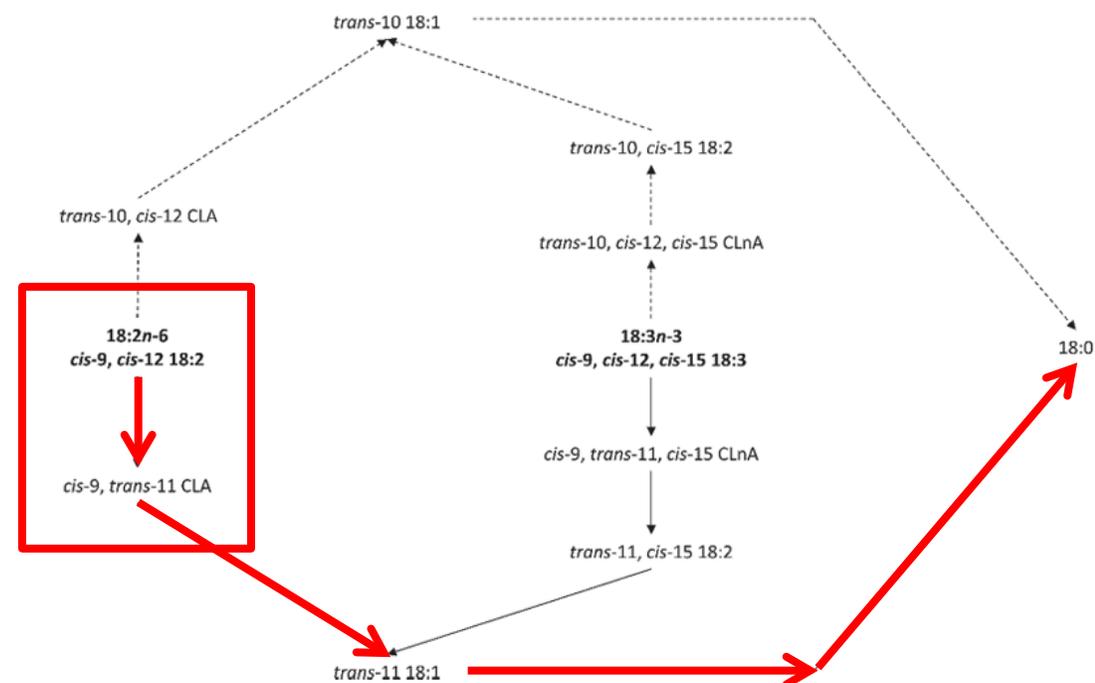
RESULTS & DISCUSSION



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- occurrence of the biohydrogenation process in the GIT of black soldier fly larvae



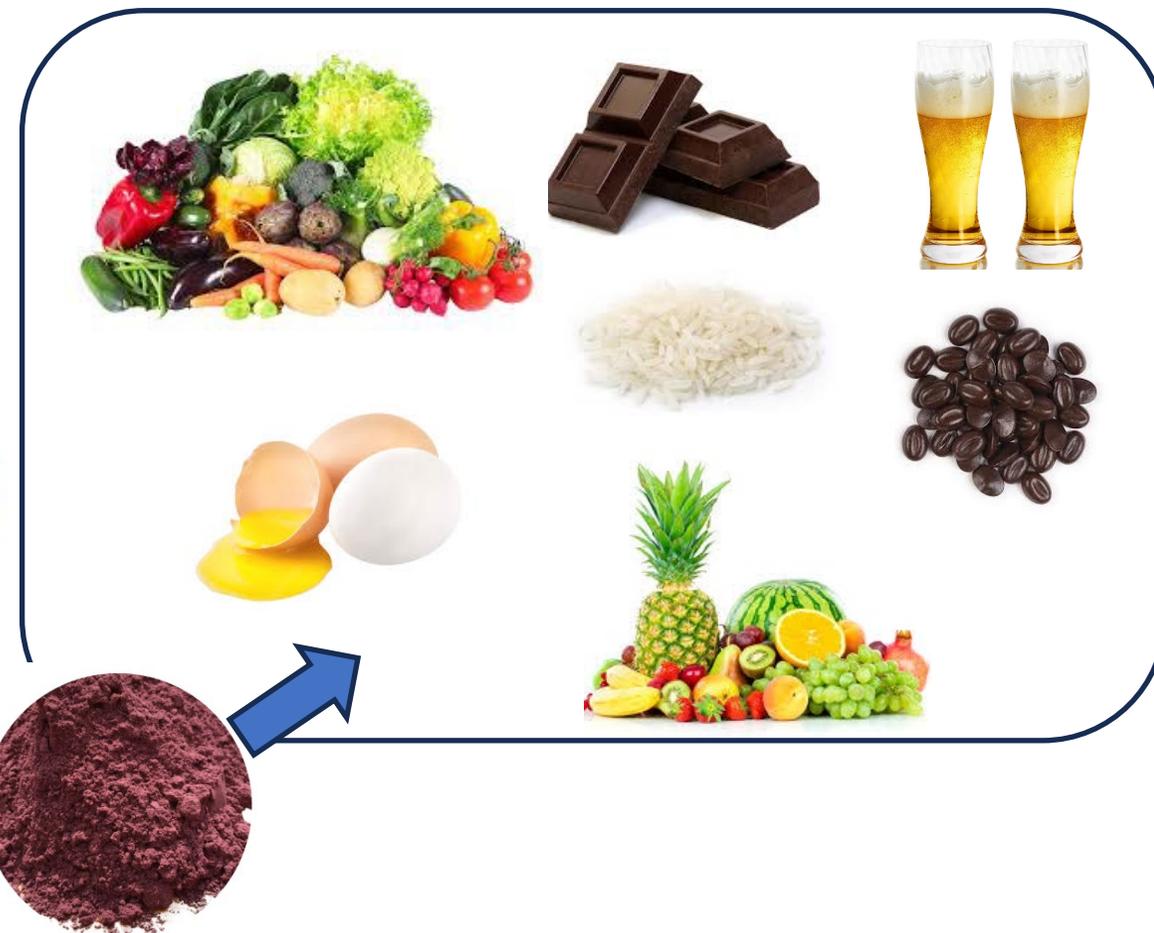
Dewanckele et al. (2020)

CONCLUSIONS

- grape pomace as rearing substrate for the improvement of the fatty acid profile of the larvae
- necessary to mix grape pomace with other organic waste and by-products to enhance the efficiency of larvae growth performance
- addition of lactic acid bacteria to rearing media to favour CLA synthesis and bioaccumulation in larvae? 
- larvae gut microbiome to understand the role of bacteria in the synthesis of fatty acids characterised by potential health-promoting effects



Image: Eke et al. (2023)





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