Fed-batch fermentation of *Yarrowia lipolytica* using defatted silkworm pupae hydrolysate: A dynamic model-based approach for high yield of lipid production

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CONTENT

01 Background

Energy shortage

Oil excavation

New oil source should be found

Microbial oils have been considered as potential feedstock for oil sources due to relatively high unicellular growth rate and rapid lipid accumulation ability. However, industrial scale implementations are presently prohibitive due to the high cost of the process, especially the cost of the medium components.

Silkworm pupae

Silkworm

Historical

Embroidery

Silk

0.5 Million tons/year

Silkworm pupae

02 Previous study

Silkworm pupae biorefinery

Silkworm chrysalis

Silkworm pupa oil

Silkworm pupa residue

Cultivate yeast

Unsaturated fatty acid[1]

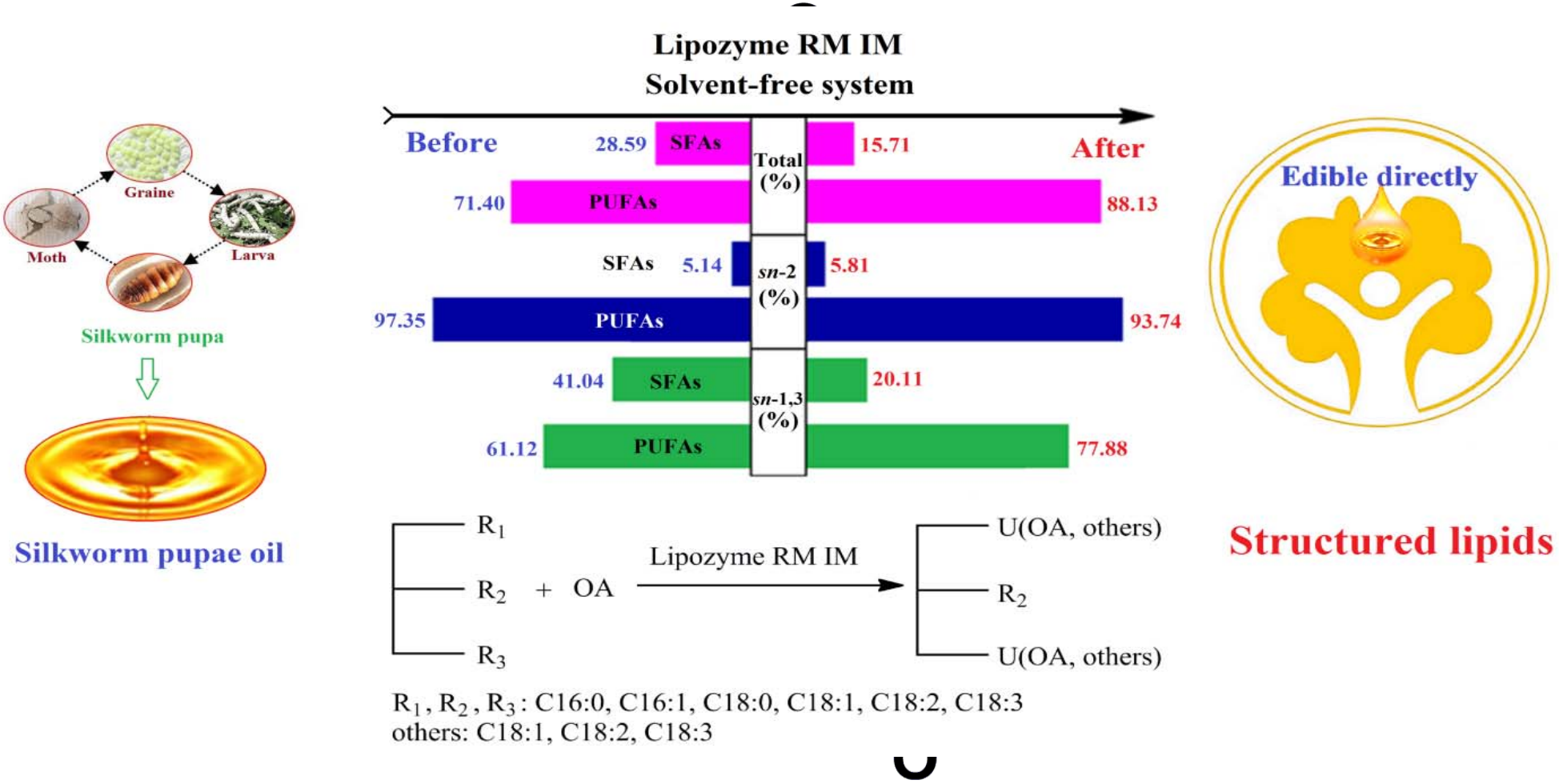
Biodiesel[2]

Structured lipids[3]

Yeast oil

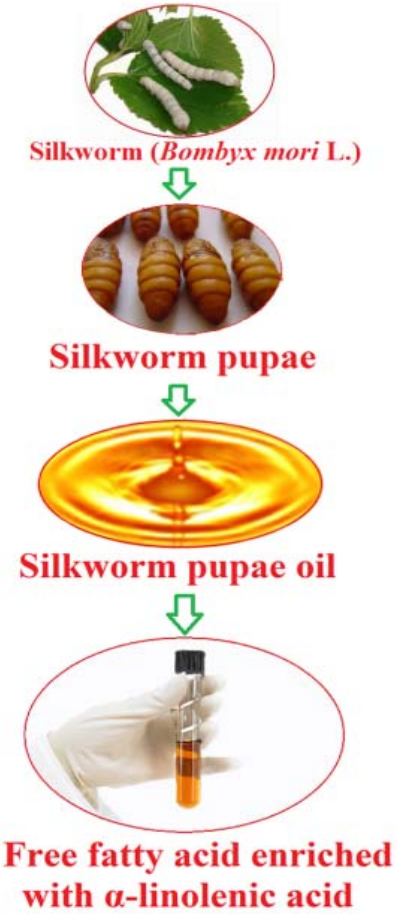
[1] Yang L I F, Siriamornpun S, Li D. Journal of Food Lipids, 2006, 13(3): 277-285.
[2]Manzano-Agugliaro F, Sanchez-Muros M J, Barroso F G, et al. Renewable and Sustainable Energy Reviews, 2012, 16(6): 3744-3753.
[3]Zhao X Y, Wang X D, Liu X, et al. European Journal of Lipid Science and Technology, 2015, 117(6): 879-889.

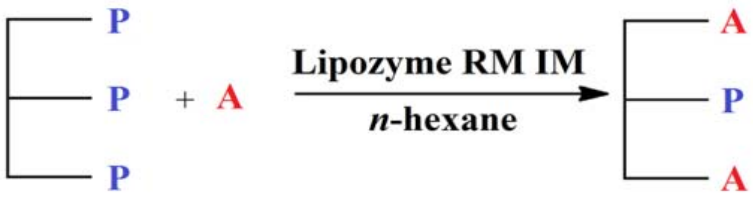
Structured lipids enriched with unsaturated fatty acids produced by enzymatic acidolysis of silkworm pupae oil using oleic acid

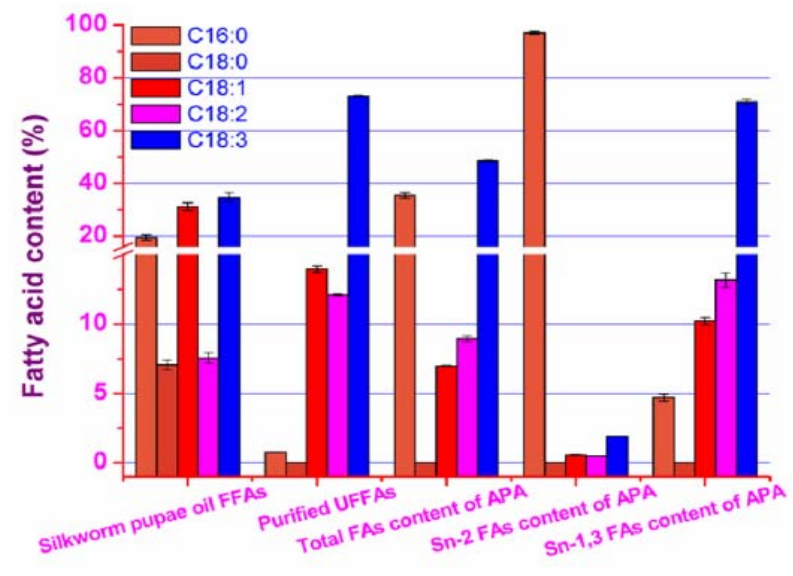


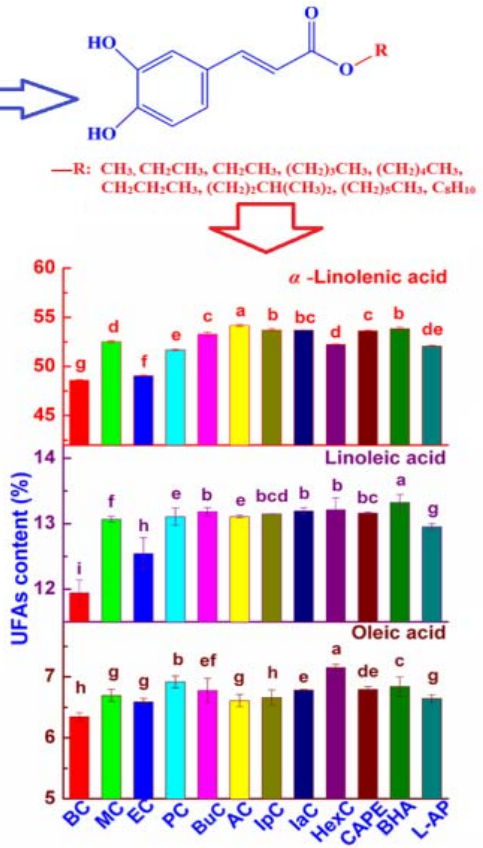
Zhao XY, Wang J*, et al. European Journal of Lipid Science and Technology. 2015, 117(6): 879-889.

APA-style human milk fat analogue from silkworm pupae oil: Enzymatic production and improving storage stability using alkyl caffeates



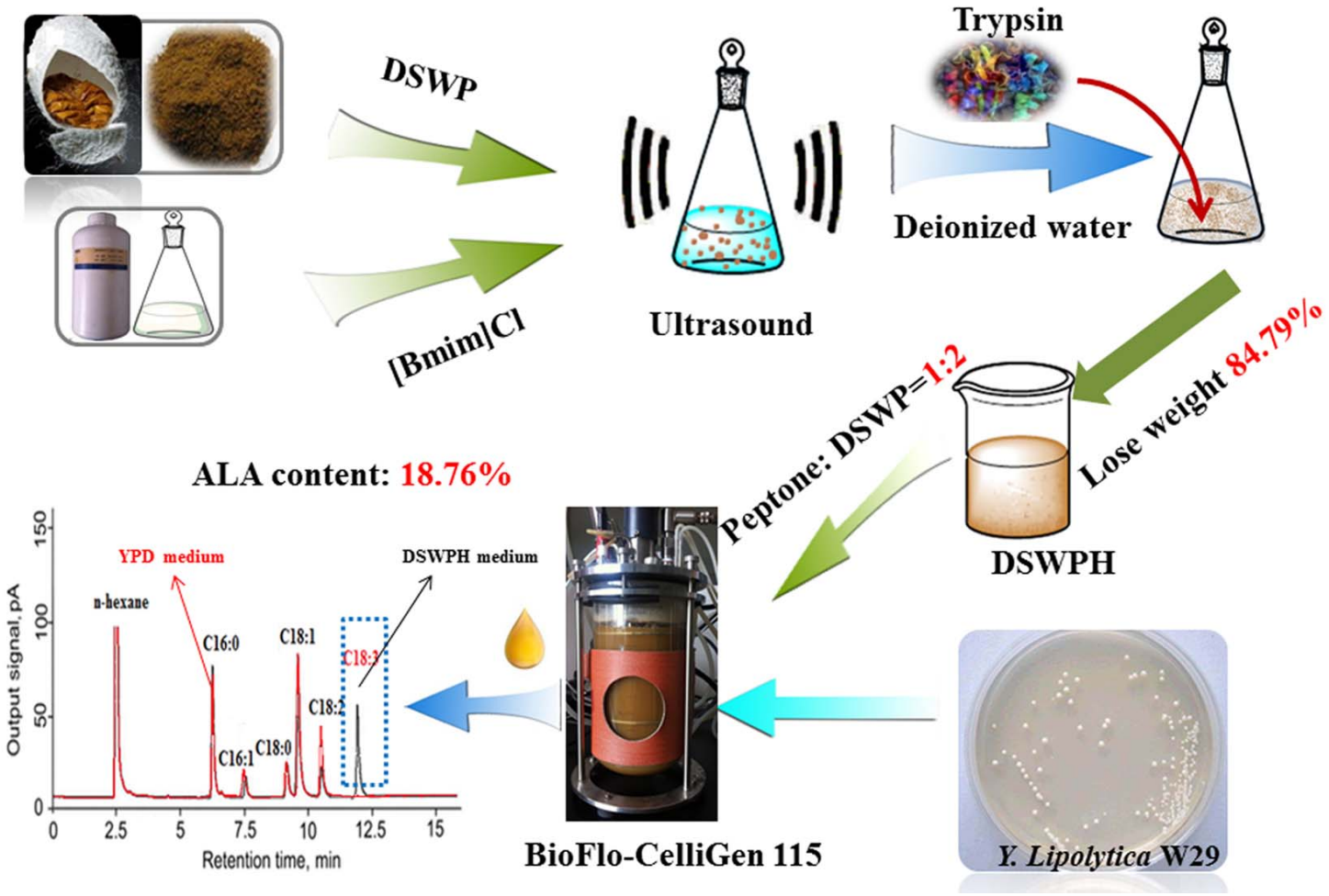






Liu X, Wang J*, et al. Scientific Reports. 2015, 5: 17909.

Converting defatted silkworm pupae by *Y. lipolytica* for enhanced lipid production



Shi XY, Wang J*, et al. European Journal of Lipid Science and Technology. 2017, 119, 1600120.

03 Present study

Microbial oils

High unicellular growth rate ?

Rapid lipid accumulation ability ?

High-value product

Problem: The price of nitrogen and carbon sources

Defatted silkworm pupae (DSWP)

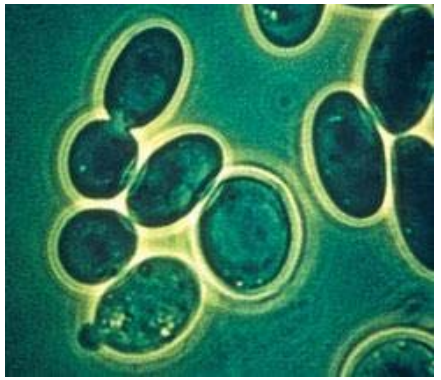
High yield

High protein content (85.2%)

hydrolysis

Soluble polypeptides

Cultivating



Yeast *Y. lipolytica* W29

The final concentration of total N is about 5.9 g L^{-1}. Considering that the total N in yeast extract is 10.0-12.5 (wt %), so per gram of yeast extract should be replaced by ca. 20 mL hydrolysate solution with a N content ranged in 5-6.25 g L^{-1}.

Characteristics of DSWPH

Different pretreatment

Microwave

Table 1 Analytical results of metals in different DSWP samples.

Metal	Control [a]	EH [b]	MWEH [c]
	(μg/g)	(μg/g)	(μg/g)
^{65}Cu	14.64±0.19	11.86±0.21	11.46±0.13
^{66}Zn	172.45±3.03	183.16±3.44	199.75±4.35
^{56}Fe	65.71±0.51	73.56±2.03	78.25±0.80
^{55}Mn	10.38±0.11	14.01±0.11	19.36±0.42
^{24}Mg	6031.31±48.51	4369.23±62.53	3635.89±72.38
^{39}K	16741.72±72.38	15332.52±45.22	13835.84±30.01
^{40}Ca	1774.40±12.18	1812.81±18.21	1927.78±17.71
^{60}Ni	0.33±0.01	0.35±0.01	0.37±0.01

[a] Condition: the material was just defatted, dried and washed by ultrapure water. And the weight loss was at 12.36%.

[b] Condition: 5 g of DSWP were mixed with 45 mL water. The neutrase was autoclaved at 55 ° C, pH 7.0 for 30 min. EH: Enzymatic hydrolysis. And the weight loss was at 59.14%.

[c] Condition: 5 g of DSWP were mixed with 45 mL water. The neutrase was autoclaved at 55 ° C, pH 7.0 for 30 min in polypropylene tubes of the microwave accelerated reaction system. MWEH: Microwave-enzymatic hydrolysis. And the weight loss was at 84.97%.

Comparison of different nitrogen source for yeast culture

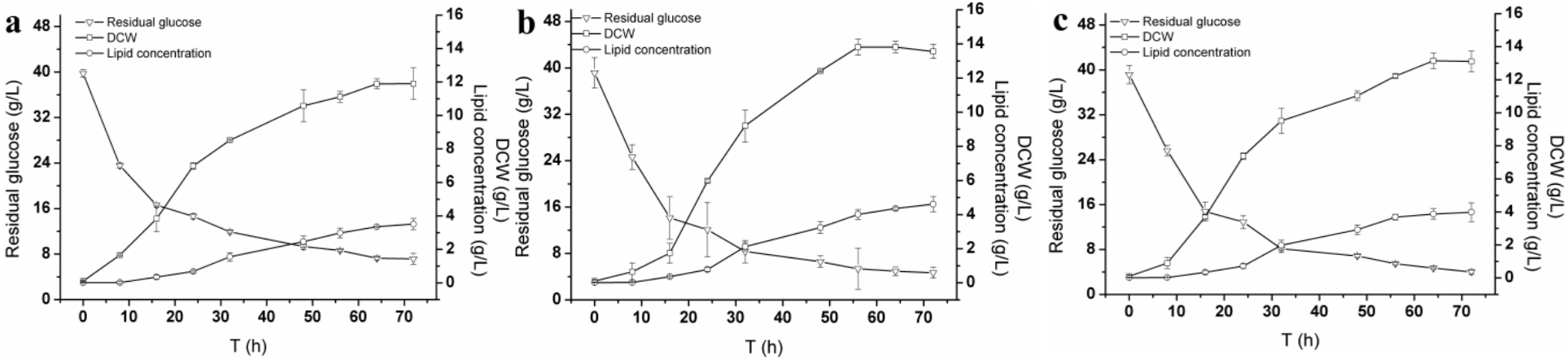


Fig. 1. Comparisons among yeast extract, DSWPH, yeast extract-DSWPH as organic nitrogen sources for cell growth and lipid production in batch cultures by *Y. lipolytica* W29. (**a. yeast-extract; b. DSWPH; c. yeast extract-DSWPH**.)

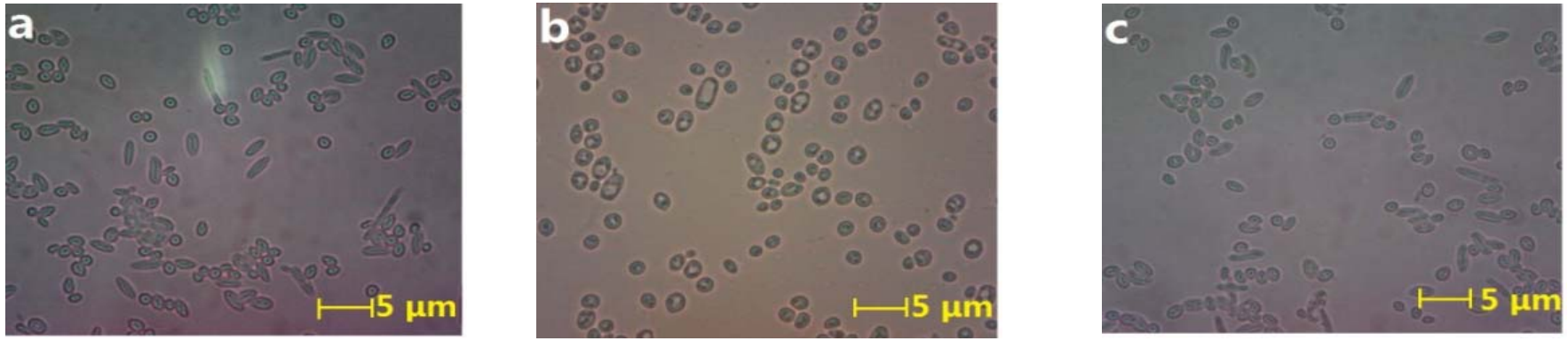
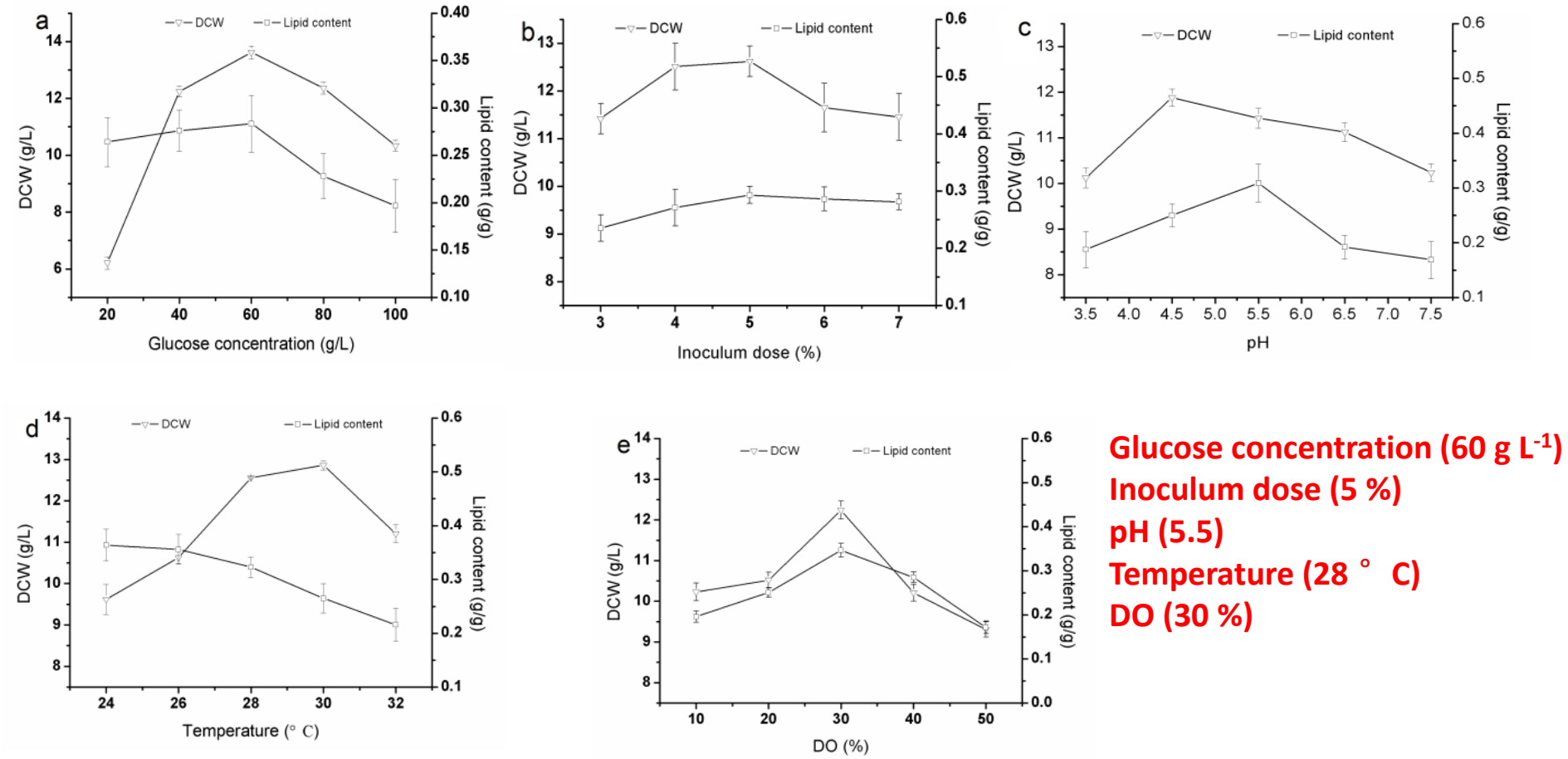


Fig. 2. Morphologies of the *Y. lipolytica* W29 grown on media made of different organic nitrogen sources. The strain was cultivated on (a) medium containing yeast extract; (b) the strain was cultivated on medium containing DSWPH; (c) the strain was cultivated on medium mixed yeast extract with DSWPH at the same nitrogen concentration. The image was captured at the end of 2 d cultivation. Conditions: initial glucose concentration 40 g/L; DO 30%; pH 5.5; incubation temperature 28 ° C.

Effects of culture conditions on cell growth and lipid accumulation



Glucose concentration (60 g L^{-1})
Inoculum dose (5 %)
pH (5.5)
Temperature (28 ° C)
DO (30 %)

Fig. 3. Growth and lipid accumulation of *Y. lipolytica* W29 under different cultivation conditions by batch culture. **(a) glucose concentration; (b) inoculum dose; (c) pH; (d) temperature; (e) DO**. Reaction conditions: (a) the inoculum was 5%, temperature was 28 ° C, pH and DO was nature; (b) the glucose concentration was 60 g/L, temperature was 28 ° C, pH and DO was nature; (c) the glucose concentration was 60 g/L, inoculum dose was 5%, culture temperature was 28 ° C.

Effects of culture conditions on the FA composition of yeast oil

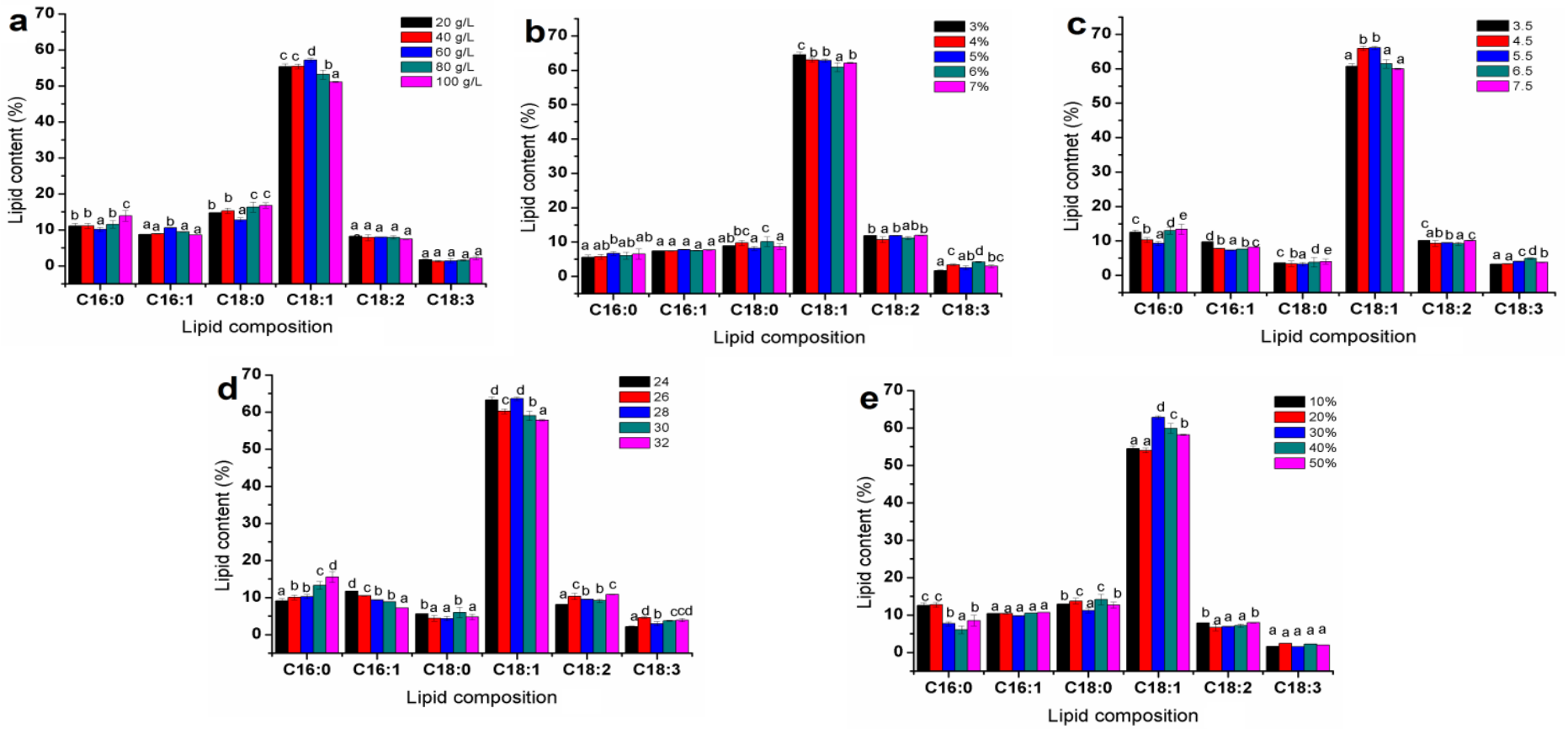


Fig. 4 Lipid profiles of *Y. lipolytica* W29 cultivated on DSWPH in batch culture. (a) glucose concentration; (b) inoculum dose; (c) pH; (d) temperature; (e) DO. Reaction conditions: (a) the inoculum was 5%, temperature was 28 ° C, pH and DO was nature; (b) the glucose concentration 60 g/L, temperature 28 ° C, pH and DO was nature; (c) the glucose concentration 60 g/L, inoculum dose 5%, culture temperature 28 ° C; (d) the glucose concentration 60 g/L, inoculum dose 5%, pH 5.5, DO was nature; (e) the glucose concentration 60 g/L, inoculum dose 5%, pH 5.5, culture temperature 28 ° C.

Table 2 Effects of nitrogen source on lipid profile and UFAs/SFAs of *Y. lipolytica* W29 in the batch fermentation.

FFAs (%, w/w)	Nitrogen source		
	Yeast extract	Yeast extract and DSWPH	DSWPH
C16:0	[a] 12.51±0.86	[b] 11.12±0.35	[b] 10.68±0.23
C16:1	[a] 10.45±0.37	[b] 8.96±0.09	[b] 8.76±0.35
C18:0	[a] 9.82±0.46	[b] 5.82±0.33	[c] 2.12±0.03
C18:1	[c] 51.74±1.06	[b] 62.42±0.42	[a] 67.30±0.96
C18:2	[a] 16.48±0.75	[b] 10.69±0.41	[c] 7.68±0.42
C18:3	[c] 0.00±0.00	[b] 1.79±0.31	[a] 3.46±0.02
UFAs/SFAs	3.48	**5.05**	**6.81**
UFAs/TFAs	78.67	**83.86**	**87.20**

[a, b, c] The mean values in the same row for *Y. lipolytica* oil TFAs culturing on different media are significantly different ($p < 0.05$). UFAs: unsaturated fatty acids; SFAs: saturated fatty acids; TFAs: total fatty acids. For the yeast *Y. lipolytica* W29, main UFAs are C16:1, C18:1, C18:2 and C18:3, main UFAs are C16:0 and C18:0.

Kinetic model for lipid fermentation

Table 3 Equations used to model growth of *Y. lipolytica* W29 on glucose and DSWPH.

Item	Kinetic equation
Microbial growth rate (Eq.2)	$\frac{dX}{dt} = \mu_m X(1 - \frac{X}{X_m})$
Lipid production rate (Eq.3)	$\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X$
Glucose consumption rate (Eq.4)	$-\frac{dS}{dt} = \frac{dX}{dt}\frac{1}{Y_{X/S}} + \frac{1}{Y_{P/S}}\frac{dP}{dt} + mX$

X: non lipid biomass concentration (g/L) at time t (h), X_0=0.106; μ_m: maximum specific growth rate (1/h), 0.191; X_m: maximum carrying capacity (g/L), 13.821; P: lipid concentration (g/L) at time t (h); α: growth-associated lipid production parameter (g/g), 0.141; β: non growth-associated lipid production parameter (g/g/h), 0.004; S: residual glucose concentration (g/L) at time t (h), S_0=39.183; $Y_{X/S}$: non lipid biomass yield coefficient (g/g), 0.345; $Y_{P/S}$: lipid yield coefficients, respectively (g/g), 0.118; m: maintenance constant, 0.010.

The model described on Fig. 5 fit the experimental glucose consumption data well with a high R^2 of 0.996.

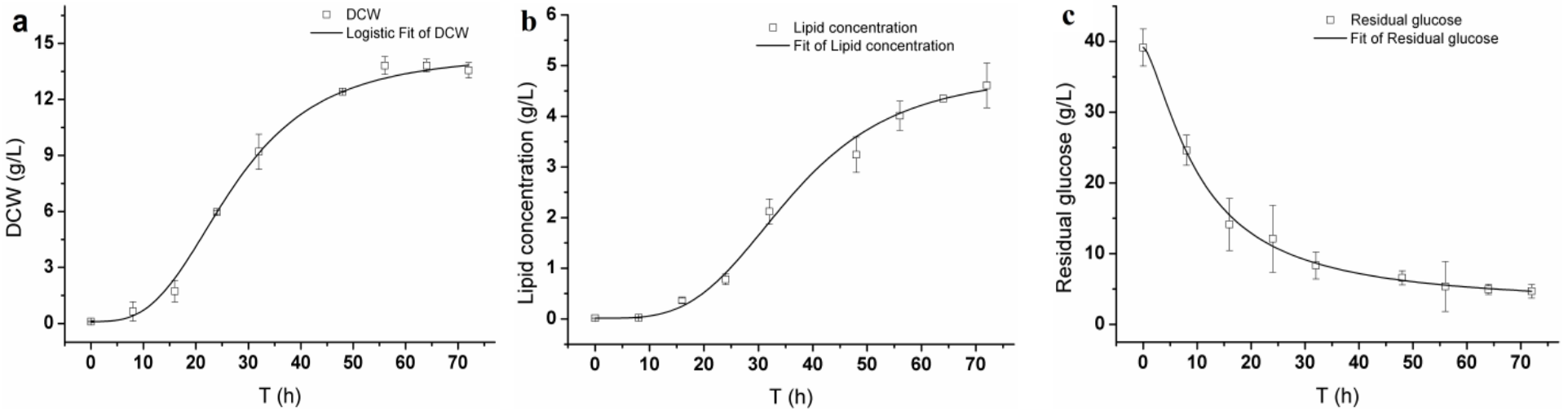


Fig. 5. Kinetics of (a) DCW, (b) lipid concentration, and (c) residual sugar in batch cultures of *Y. lipoltytica* W29 cultured on DSWPH medium with time-corrected Luedeking-Piret models. Culture conditions: initial glucose concentration 40 g/L; DO was 30% of saturation; pH 5.5; incubation temperature 28 ° C.

Table 4 Applications of *Y. lipolytica* W29 use different waste sources as inexpensive alternative substrates.

Y. lipolytica	Medium	Mode culture	DCW (g/L)	Lipid content (%, w/w)	Fermentation time (h)	Lipid productivity (mg/L/d)	Refs
JMY4086	Molasses and crude glycerol	Continuous	60	31	450	430	[41][a]
LGAM S(7)1	Industrial glycerol	Continuous	8.14	43	168	120	[40][b]
MUCL 28849	Volatile fatty acids	Two-stage fed-batch	31	40	60	206	[3][c]
W29	Glucose and olive mill wastewater-based media	Flask	6.8	27.9	72	26	[7]
-	Non-detoxified liquid wheat straw hydrolysate	Flask	7.8	4.6	144	2	[6]
W29	Glucose and DSWPH	Batch	13.9	34.6	72	67	[42]
W29	Glucose and DSWPH	Fed-batch	38.3	28.8	96	115	This study

[a] The strain was cultured for 450 h in a chemostat containing a nitrogen-limited medium (dilution rate of 0.01 h^{-1}, 250 g/L crude glycerol), and volumetric lipid productivity was 0.43 g/L/h and biomass yield was 60 g CDW/L.

[b] This was produced in highly aeracted continuous. Lipid production was favoured at low specific dilution rate whilst fat-free material yield increased over the whole range of specific dilution rate.

[c] The strain was initially grown on glucose or glycerol as carbon source then sequential additions of glucose under nitrogen limiting conditions were performed after glucose or glycerol exhaustion.

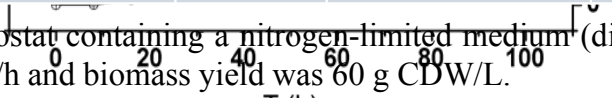


Fig. 6. Time course of fed-batch fermentation on residual glucose, DCW, and lipid concentration of the *Y. lipoltytica* W29 cultivated in the 3.0 L fermentor with a 2.0 L DSWPH medium. The feeding medium was containing 160 g/L glucose and 40 g/L DSWPH. The feeding was started after 24 h and ended at 71 h.

04 Conclusion

1. Lipid production by *Y. lipolytica* W29 in fed-batch mode was investigated by using low-cost substitutable defatted silkworm pupae hydrolysate (DSWPH) as a feedstock.

2. Three media (yeast extract, DSWPH, yeast extract-DSWPH as N sources) were investigated in a batch fermentation process. The DSWPH medium displayed the optimal lipid accumulation ability with a lipid yield raised by 16.13%, a ratio of unsaturated fatty acids *vs.* saturated fatty acids inproved by 0.96-fold, and a ratio of unsaturated fatty acids in total fatty acids increased to 87.23%.

3. The mathematical equations based on experimental data provided a good description of temporal variations such as dry cell weight, glucose consumption, and product formation in lipid fermentation. The results showed that the Luedeking–Piret type equation successfully described glucose consumption and lipid accumulation in the batch culture process.

4. A fed-batch fermentation system was designed based on the model prediction. In the lag phase, rapid biomass growth and lipid accumulation were sequentially achieved with the adjustment of temperature, pH, and dissolved oxygen. Finally, the maximum biomass and lipid productivity were 24.01 g/L and 2.76 g/L/d, respectively.

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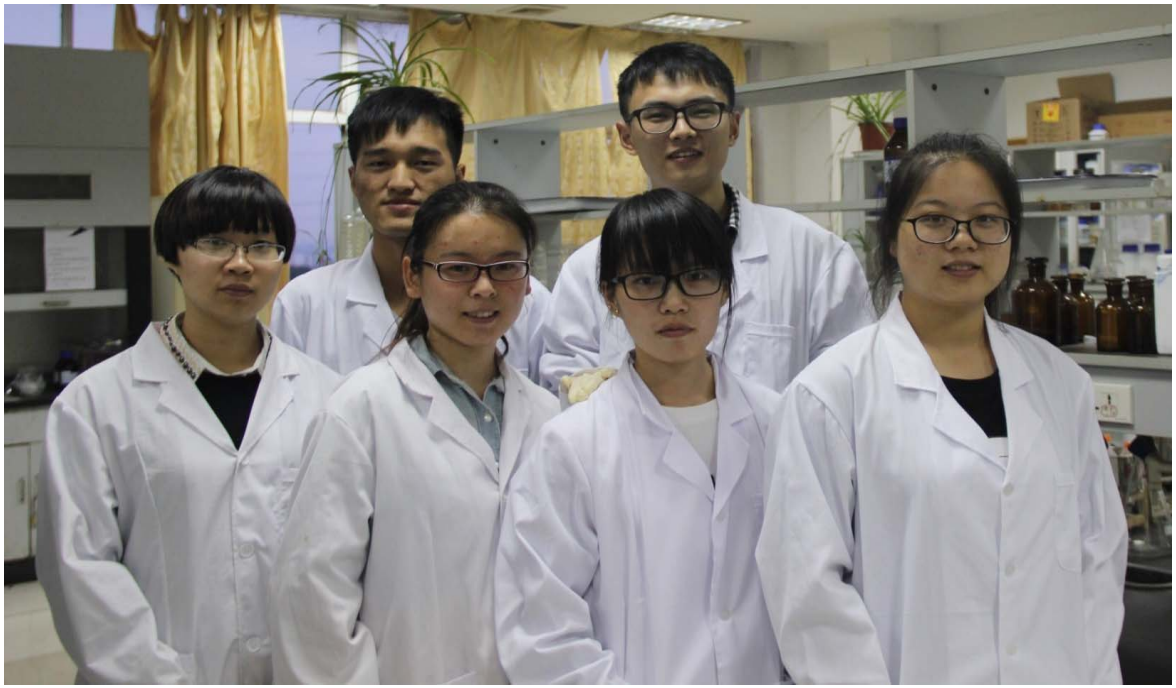

THANK YOU