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Recycling of orange waste for single cell protein production and the synergistic and antagonistic effects on production quality

Yue-Ming Zhou [a, b], You-Peng Chen [a, *], Jin-Song Guo [a, **], Yu Shen [b, c], Peng Yan [a, b], Ji-Xiang Yang [b]

[a] Key Laboratory of the Three Gorges Reservoir Region's Eco-Environments of MOE, Chongqing University, Chongqing, 400045, China
[b] Key Laboratory of Reservoir Aquatic Environment of CAS, Chongqing Institute of Green and Intelligent Technology, Chinese Academy of Sciences, Chongqing, 400714, China
[c] National Base of International Science and Technology Cooperation for Intelligent Manufacturing Service, Chongqing Technology and Business University, Chongqing, 400067, China

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ABSTRACT

The daily output of orange residue from the orange juice production enterprise in the Three Gorges Reservoir Area (TGRA) of China is approximately 100 t/d, which seriously pollutes the environment of the TGRA. The key challenge of handling this waste is maximizing profitability. In this study, high-protein feed with low levels of crude fiber and pectin was produced by solid fermentation of orange waste. The synergistic and antagonistic effects of microorganisms on one another significantly influenced the quality of single cell protein (SCP) feed. The added true protein (ATP) content increased gradually as pectin degradation (PD) and crude fiber degradation (CFD) were enhanced because of the synergistic effects. However, ATP decreased because of antagonistic effects as PD and CFD were increased beyond certain values. *Aspergillus oryzae* (*A. oryzae*) and *Trichoderma koningii* (*T. koningii*) mutually promoted each other, but the growth of *Candida tropicalis* was inhibited by *A. oryzae* and *T. koningii* as polygalacturonase and carboxymethyl cellulase accumulated. Synergistic and antagonistic effects existed simultaneously during microorganic fermentation of orange wastes. In large-scale fermentation, ATP, PD, and CFD were increased by 14.20%, 15.80%, and 9.15%, respectively, in comparison with the flask test. The profit achieved by reusing orange waste in the Chongshou Agricultural Park as SCP feed was calculated to be 48500 USD per year, whereas the cost of disposing of the orange waste was 7560 USD. This study provides insight into how microorganic synergistic and antagonistic effects influence the quality of SCP feed and provides a potential route for recycling agricultural waste into valuable materials.

1. Introduction

China is the foremost orange producer in the world. In 2014, the orange planting area of China was 2.211×10^{10} m^2, and the annual output was 2.75×10^{10} kg (Shan, 2014). Oranges are consumed worldwide in the forms of pulp, peel and juice. Approximately 8×10^{9}-2×10^{10} kg of solid and liquid residue is produced as waste during orange processing every year (Tripodo et al., 2004; Rezzadori et al., 2012). In most instances, this enormous amount of waste is scattered on soil in areas adjacent to the production facility, incinerated, placed in a land fill, or used as raw material for animal feed production (Martín et al., 2010; Patsalou et al., 2017). However, incineration of orange waste was found to lead to highly polluted surface and ground water with altered chemical oxygen demand and biological oxygen demand, as well as polluted soil (Rezzadori et al., 2012; Braddock, 1995).

The term SCP refers to dead, dry cells of microorganisms such as yeast, bacteria, fungi and algae, which are utilized as a protein supplement in foods for humans or animal feed (Ofodile et al., 2011; Mahmood, 2012). SCP is made up of carbohydrates, fats, vitamins, minerals, and other cellular constituents (Mondal et al., 2012), and it can be produced from relatively inexpensive material or waste material (Santamaría-Fernandez et al., 2017).

* Corresponding author. 266 Fangzheng Avenue, Beibei, Chongqing, 400714, China.
** Corresponding author. 266 Fangzheng Avenue, Beibei, Chongqing, 400714, China.
E-mail addresses: ypchen@cqu.edu.cn (Y.-P. Chen), guo0768@cqu.edu.cn (J.-S. Guo).

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Orange waste is an attractive nutrient source for microbial communities (López et al., 2010; Kantifedaki et al., 2018). Citrus peel waste from the orange industry in the Three Gorges Reservoir Area (TGRA) of China could be used as a raw material for SCP, which could be used in feed for the poultry industry, thus reusing orange waste with considerable economic and social benefits. However, orange waste contains insoluble components such as cellulose (9.21% dry weight), hemicelluloses (10.5% dry weight), and pectin (42.5% dry weight), which lead to animal feed with poor palatability, a low absorption rate, and rapid spoilage (Wilkins et al., 2007). Therefore, methods of obtaining high-protein feed with low levels of pectin and crude fiber are essential for effective utilization of orange waste in animal feed.

Mixed microorganism communities play a key role in determining the taste, quality, and safety of a wide range of foods, including some types of animal feed (Sieuwerts et al., 2008). The SCP feed fermented by mixed strains was widely used in developing countries such as India (Mondal et al., 2012), Brazil (Rezzadori et al., 2012), Pakistan (Sadiq et al., 2014) and China (Shan, 2014) for animal food supply in the last 30 years. *Aspergillus niger* (*A. niger*) and *Saccharomyces cerevisiae* (*S. cerevisiae*) were evaluated for the production of SCP feed using orange peels as sole carbon source. For *S. cerevisiae*, protein contents were increased to more than 22.06% and *A. niger* were increased to more than 27.15% (Sadiq et al., 2014). Nishio employed *Debaryomyces hansenii* and *Rhodotorula glutinis HUT 7530* into SCP biomass production. After fermentation, the protein content of SCP product which inoculated mixed yeasts was 11% higher than that inoculated single strain (Nishio and Nagai, 1981). Moreover, *A. niger*, *A. oryzae* and *Endomycopsis fibuligera* were inoculated to mixed substrates with the ratio of 2:3:1 for SCP fermentation. In the end, the crude protein content were increased to 34.40% compared to 10.37% at the initial stage (Yin et al., 2009). Mixed microorganisms are more suitable for SCP fermentation, however, the interaction among strains are still not clear. Previous studies of the synergistic and antagonistic effects of microorganisms in orange waste have primarily assessed the interactions between essential oils, flavonoids, and pathogenic microorganisms (Mandalari et al., 2007). However, comprehensive studies of the influence of synergistic and antagonistic effects in mixed communities of microorganisms on feed quality have not been performed.

The first aims of this study were to develop a method of reusing orange waste in high-protein animal feed with low levels of pectin and crude fiber, optimize the composition of functional strains in the mixed microorganism community used to produce the feed. Secondly, the influence of microorganic synergistic and antagonistic effects on feed quality was systematically explored and verified by the analysis of polygalacturonase (PGase) and carboxymethyl cellulase (CMCase) activity. Thirdly, the colony counting of mold and yeast were also explored to verify the existence of synergistic and antagonistic effects. Finally, the improved SCP feed quality and the economic benefit of large-scale fermentation of orange waste in the TGRA were analyzed.

2. Materials and methods

2.1. Microorganisms

The 3 strains used in this study were purchased from the China Center of Industrial Culture Collection and subcultured every week. *A. oryzae* degrades pectin and produces proteins (Han et al., 2016), *Candida tropicalis* (*C. tropicalis*) produces proteins (Kwon et al., 2006), and *T. koningii* degrades cellulose and produces proteins (Wood and Mccrae, 1982).

The *C. tropicalis* culture medium contained 10.0 g/L yeast extract, 20.0 g/L peptone, and 20.0 g/L glucose. The culture medium for *A. oryzae and T. koningii* contained 200.0 g/L potato and 20.0 g/L glucose. The strains were each cultivated for 4 d at 33 °C to obtain spore concentrations of approximately 1×10^6 CFU/mL.

Table 1
Composition of substrates (% dry weight). The mixed substrate was 20% (dry weight) soybean cake and 80% (dry weight) orange waste.

Composition	True protein	Crude fiber	Pectin	Ash	Moisture
Soybean cake	31.90	8.68	3.11	7.70	9.50
Orange waste	8.54	29.40	23.47	4.51	76.83
Mixed substrate	13.11	26.32	18.52	5.07	63.02

2.2. Substrates

Due to the low nitrogen content of orange waste, additional nitrogen-rich material was added to the substrate in the form of soybean cake. The mixed substrate contained 20% (dry weight) soybean cake and 80% (dry weight) orange waste (Table 1). The orange waste and soybean cake were collected from an orchard located in Changshou Agricultural Park in TGRA (Chongqing, China), oven-dried at 60–65 °C, crushed, and passed through a sifter. Prior to use, the orange waste and soybean cake were mixed and sterilized at 121 °C for 20 min.

2.3. Fermentation with a single strain

The experiments were carried out in conical flasks with 5 g of the dry mixed substrate. The moisture content of the substrate was adjusted by adding sterile water. Next, 2.5 mL inoculums of each strain were aseptically transferred into the substrate, which was mixed well. The control groups were not inoculated with a microorganism. The fermentation conditions were as follows: 0.5 mL/g (dry weight) inoculum concentration, 70% (w/w) moisture rate, 84 h fermentation time, 33 °C.

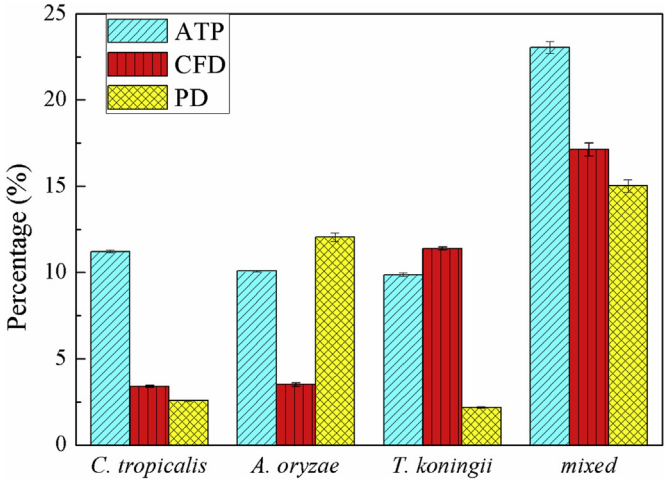


Fig. 1. Effects of 3 single strains and mixed strains. The fermentation conditions of a single strain were as follows: 0.5 mL/g (dry weight) inoculum concentration, 70% (w/w) moisture rate, 84 h fermentation time, 33 °C. The mixed strains were *C. tropicalis, A. oryzae* and *T. koningii* (0.2 mL/g, 0.2 mL/g and 0.18 mL/g, respectively). The fermentation conditions of mixed strains were as follows: 70% (w/w) moisture rate, 84 h fermentation time, 33 °C.

2.4. Optimization of the ratio of strains in the mixed microorganism community

The optimal mixed strain ratio was determined by response surface method (RSM) analysis with ATP as the response value. The experiments utilized an orthogonal Box-Behnken design, with the low level set to 0.1 mL/g and the high level set to 0.2 mL/g (Table A). The data were analyzed by quadratic regression, and the degree of model fitting was evaluated by ANOVA with Design-Expert (Version 8.0.6, Stat-Ease Inc., USA).

2.5. Lab-scale fermentation of single cell protein feed in flask

The optimal mixed strain ratio determined in the analysis described above was applied. The total mixed substrate used in this experiment was 20 g, the moisture content was 70%, and the temperature was 33 °C.

2.6. Large-scale production of single cell protein feed in fermentation tank

Large-scale production of SCP feed was performed in a 50 L fermentation tank (sy0-Terrafors, China). First, 30 L of the mixed substrate (70% moisture rate, w/w) was transferred into the tank and sterilized at 121 °C for 20 min. Prior to fermentation, seed solutions of *C. tropicalis*, *A. oryzae* and *T. koningii* were inoculated into the tank at concentrations of 3.0 L/kg, 3.0 L/kg, and 2.7 L/kg, respectively. The fermentation conditions were as follows: 33 °C, DO 25%, pH 5.52 (natural pH), agitation speed 50 rpm. Sampling was performed at 84 h, 96 h, and 108 h to assay true protein, PGase, CMCase, pectin, crude fiber and ash.

2.7. Polygalacturonase and carboxymethyl cellulase activity

A 10 g sample was mixed with 90 mL sterile water, shaken at 120 rpm for 30 min, and centrifuged at 8000 rpm for 10 min. The supernatant was collected as a crude enzyme sample. Pectin can be hydrolyzed to D-galacturonic acid by PGase. To determine the activity of PGase, 0.2 mL of crude enzyme solution and 1.8 mL of citrus pectin solution (0.40% citrus pectin in 50 mM sodium acetate buffer, pH = 5.0) were incubated at 33 °C for 30 min, then terminated by the addition of 2 mL of dinitrosalicylic acid. PGase activity was determined by measuring the D-galacturonic acid formation rate using colorimetric method (Somogyi, 1952; Solis-Pereira et al., 1993). Crude fiber can be hydrolyzed to glucose by CMCase. To determine the activity of CMCase, 0.5 mL of crude enzyme solution and 2.0 mL of sodium carboxymethyl cellulose solution (0.63% sodium carboxymethyl cellulose in 50 mM sodium acetate buffer, pH = 5.0) were incubated at 33 °C for 30 min, then terminated by the addition of 3 mL of dinitrosalicylic acid. CMCase activity was determined by measuring the glucose formation rate using colorimetric method (Ghose, 1987; Wilkins et al., 2007).

2.8. Microbial quantity after fermentation

One gram of fermented feed was shaken with 100 mL sterile water for 15 min, after which 1 mL of the suspension was diluted and used for mold (*A. oryzae* and *T. koningii*) and yeast (*C. tropicalis*) counting according to the dilution plate method. The counting medium was Rose Bengal Agar Medium (Abe et al., 2008).

2.9. Other analytical methods

Temperature, moisture content, true protein, crude fiber, pectin and ash were analyzed in triplicate. The moisture content of the samples was determined by oven-drying at 105 °C for 24 h. Ash content was determined by igniting the fruit waste in a muffle furnace at 550 °C as previously described (Pearson, 1981). Pectin content was determined by sulfuric acid-carbazole colorimetry (Pang et al., 2012). TP content was determined by the Kjeldahl method (K−1100F, China) and calculated as total N multiplied by 6.25 (AOAC, 2006). Crude fiber was determined using improved AOAC method and Van-Soest method by separating out protein, fat, sugar and inorganic salt from dry SCP production (Van-Soest et al., 1991; AOAC, 2006).

2.10. Statistical analysis

Partial correlation analysis was performed using the PASW software package (Statistics 18 Inc., USA). The figures were created using OriginPro 8.0 software (USA). ATP, CFD and PD were calculated as follows:

$$A = (C_T - C_0) / C_0 \times 100\% \quad (1)$$

Where A is the added rate of true protein, (%); C_T is the content of true protein over time; C_0 is the initial content of true protein.

$$D = (W_0 - W_T) / W_0 \times 100\% \quad (2)$$

Where D is the degradation rate of crude fiber or pectin, (%); W_T is the content of crude fiber or pectin over time; W_0 is the initial content of crude fiber or pectin.

One unit (U) of PGase activity was defined as the amount of

Table 2
ANOVA for the response surface quadratic model. Analysis of the Box-Behnken experiment.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	44.93	9	4.99	99.21	<0.0001	significant
A- *C. tropicalis*	20.00	1	20.00	397.51	<0.0001	
B– *A. oryzae*	11.88	1	11.88	236.14	<0.0001	
C– *T. koningii*	3.62	1	3.62	71.90	<0.0001	
AB	0.64	1	0.64	12.72	0.0091	
AC	0.57	1	0.57	11.33	0.0120	
BC	0.62	1	0.62	12.25	0.0100	
A^2	2.90	1	2.90	57.57	0.0001	
B^2	2.18	1	2.18	43.32	0.0003	
C^2	1.74	1	1.74	34.49	0.0006	
Residual	0.35	7	0.050			
Lack of Fit	0.34	3	0.11	30.57	0.0032	significant
Pure Error	0.015	4	3.680×10^{-3}			
Cor Total	45.28	16				

enzyme that released 1 μmol of D-galacturonic acid per minute. One U of CMCase activity was defined as the amount of enzyme that released 1 μmol of glucose per minute (Wilkins et al., 2007). The enzyme activity were calculated as follows:

$$X_{PGase} = m_1 / (M_1 \times t) \times 1000 \times n_1 \quad (3)$$

Where X_{PGase} is the activity of PGase (U/g); m_1 is the corresponding content of D-galacturonic acid which is calculated according to the

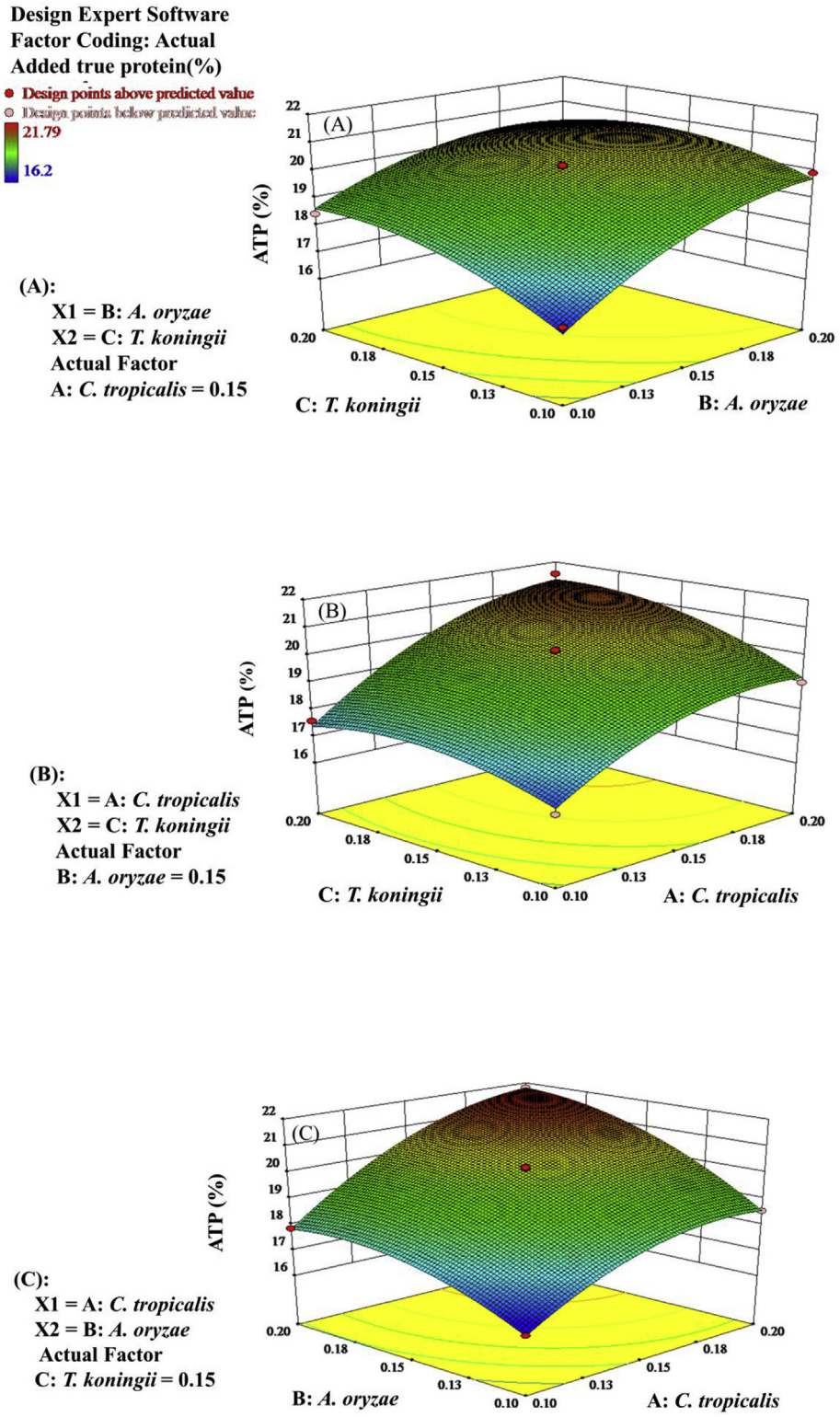


Fig. 2. Response surface plot for the interaction between *C. tropicalis*, *A. oryzae* and *T. koningii* on ATP. The experiments utilized an orthogonal Box-Behnken design, with the low level set to 0.1 mL/g and the high level set to 0.2 mL/g. (A) Interaction between *T. koningii* and *C. tropicalis*; (B) Interaction between *T. koningii* and *A. oryzae*; (C) Interaction between *A. oryzae* and *C. tropicalis*.

Table 3
Correlational analysis of added true protein (ATP), crude fiber degradation (CFD), and pectin degradation (PD).

Variables		ATP	CFD	PD
ATP	Pearson correlation	1.000	.903[a]	.919[a]
	Significance (2-tailed)	–	.000	.000
	N	42	42	42
CFD	correlation	.903[a]	1	.987[a]
	Significance (2-tailed)	.000		.000
	N	42	42	42
PD	correlation	.919[a]	0.987[a]	1
	Significance (2-tailed)	.000	.000	–
	N	42	42	42

[a] Correlation is significant (2-tailed).

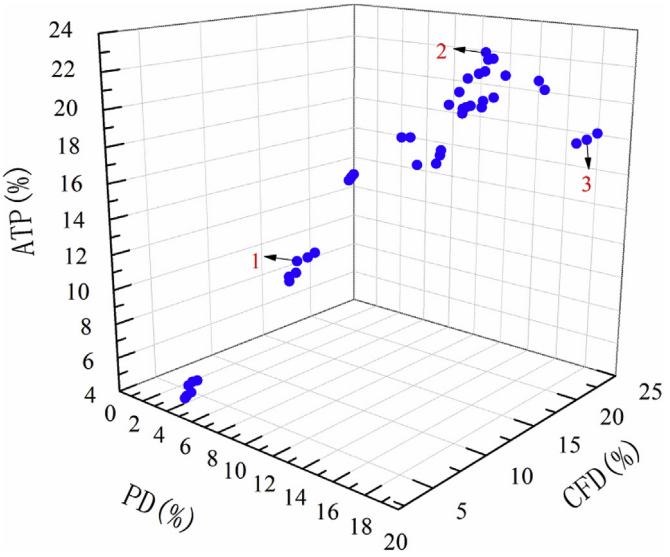


Fig. 3. The relationship between ATP, CFD and PD.

standard curve equation (mg); M_1 is the molar mass of D-galacturonic acid, 194.14 g/mol; t is the time of enzymatic hydrolysis reaction, 30 min; 1000 is the conversion factor from mmol to μmol; n_1 is the dilution ratio of sample, 50 times.

$$X_{CMCase} = m_2 / (M_2 \times t) \times 1000 \times n_2 \quad (4)$$

Where X_{CMCase} is the activity of CMCase (U/g); m_2 is the corresponding content of glucose which is calculated according to the standard curve equation (mg); M_1 is the molar mass of glucose, 180.2 g/mol; t is the time of enzymatic hydrolysis reaction, 30 min; 1000 is the conversion factor from mmol to μmol; n_2 is the dilution ratio of sample, 20 times.

3. Results and discussion

3.1. Characteristic of strains

3.1.1. Fermentation characteristics of a single strain

The results obtained using each of the 3 strains separately are shown in Fig. 1. *C. tropicalis* produced a higher protein yield in comparison with the other two strains, indicating that its main function was protein production. The PD rate of *A. oryzae* was far greater than that of *C. tropicalis* or *T. koningii*, demonstrating its capacity for pectin degradation. *T. koningii* had a CFD rate greater than that of *C. tropicalis* and *A. oryzae*, suggesting that its main role was degradation of crude fiber. These results demonstrate that the three tested strains should be used together to obtain SCP with high protein content.

3.1.2. Optimization of the mixed strain ratio

Eq. (5) shows the relationship between the 3 tested strains and ATP. The parameters of the equation were obtained by regression analysis of the experimental data. In Eq. (5), *Y* was the ATP concentration, whereas X_1, X_2, and X_3 were the concentrations of *C. tropicalis, A. oryzae* and *T. koningii*, respectively.

$$Y = 20.14 + 1.58X_1 + 1.22X_2 + 0.67X_3 + 0.40X_1X_2 + 0.38X_1X_3 - 0.39X_2X_3 - 0.83X_1^2 - 0.72X_2^2 - 0.64 \times \frac{2}{3} \quad (5)$$

The F-value from the ANOVA implied that the model was significant, because there was found to be only a 0.01% chance that an F-value this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicated that the model terms were significant (Table 2).

The relationships between ATP and different combinations of strains are shown in Fig. 2. Utilization of all 3 strains dramatically increased the true protein content compared with that obtained using pure cultures of each strain alone. The optimum concentrations of *C. tropicalis, A. oryzae* and *T. koningii* were calculated to be 0.2 mL/g, 0.2 mL/g and 0.18 mL/g, respectively. The predicted ATP percentages were all 21.96%, which were validated by actual experiments (22.31%, 21.20%, 21.61%, 20.79%, 20.79%, 21.38%) under the same conditions. The results of these experiments confirmed that the difference between the predicted and experimental average values was only 0.61%, and the relative standard deviation of the experimental values was 2.69%. Both of these findings indicated the reliability of the RSM analysis.

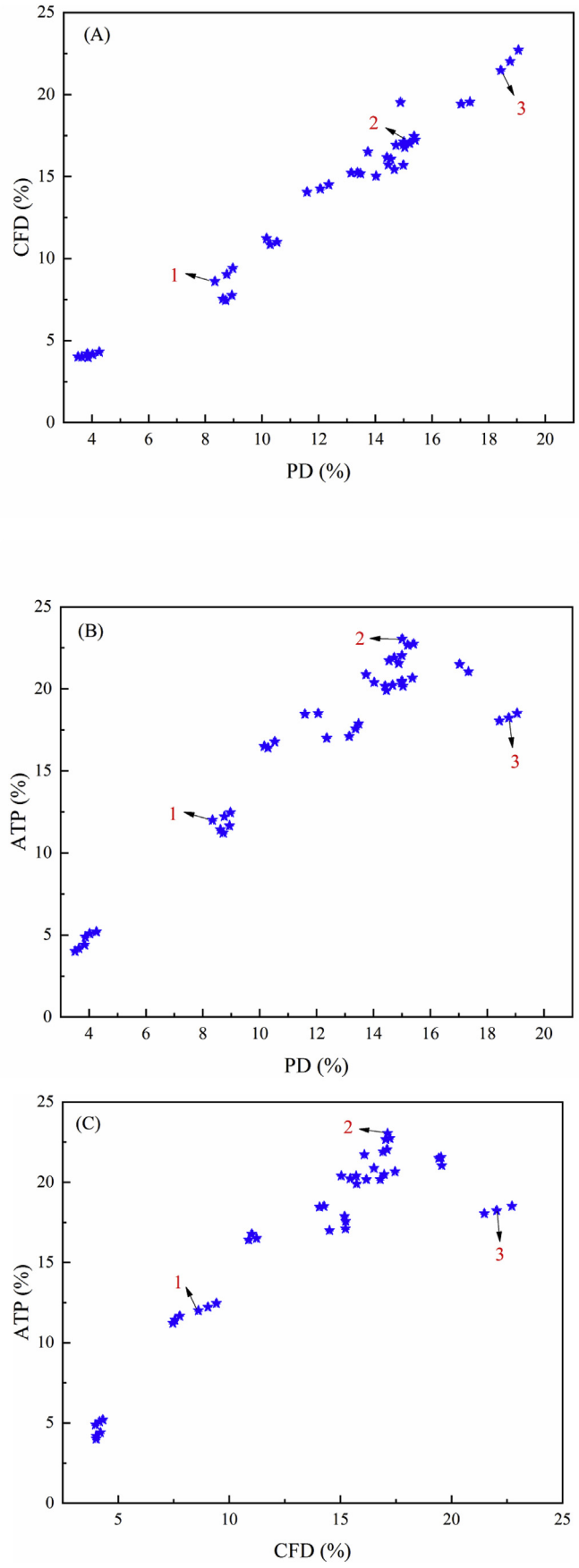
Furthermore, the mixed microorganism community showed efficient CFD, PD and protein production (Fig. 1). In comparison with the results obtained using a single strain, ATP was increased by 11.79%, CFD was increased by 5.67%, and PD was increased by 3.00%. These results indicate that a microbial synergistic effect existed among the different functional strains during mixed fermentation.

3.2. The relationship between A. oryzae, C. tropicalis and T. koningii during lab-scale fermentation

3.2.1. Synergistic effect of A. oryzae, C. tropicalis and T. koningii during lab-scale fermentation

The results of the correlation experiments are shown in Table 3. The correlation coefficients of ATP and CFD, ATP and PD, and PD and CFD were all greater than 0.903, suggesting that these three measurements were closely related.

The results shown in Fig. 3 illustrate the relationship among ATP, CFD and PD. As shown in Fig. 4 (A), CFD increased as PD increased throughout its range. As shown in Fig. 4 (B) and Fig. 4 (C), ATP



increased as CFD and PD increased when PD was lower than 15.01% and CFD was lower than 17.12%, confirming that a synergistic effect was produced by the community of mixed strains. The maximum true protein content was 16.13%, whereas the pectin content was 15.74%, and the crude fiber content was 21.81%. These results suggest that SCP production, pectin degradation, and cellulose degradation occurred simultaneously and stably because of cooperation by *A. oryzae*, *C. tropicalis* and *T. koningii*.

3.2.2. Antagonistic effect of A. oryzae, C. tropicalis and T. koningii during lab-scale fermentation

However, as shown in Fig. 4 (B) and Fig. 4 (C), ATP decreased when PD was greater than 15.01% and CFD was greater than 17.12%. In brief, degradation of crude fiber and pectin had an antagonistic effect on protein production. The degradation rates of crude fiber and pectin are determined by enzymes (Romaní et al., 2006), so the antagonistic effect on SCP production was probably due to active crude fiber enzymes (cellulase, hemicellulose and lignin enzymes) (Tripodo et al., 2004) and pectinase (Gregorio et al., 2002), which accumulated in the flask and inhibited SCP production. Among the tested strains, crude fiber enzymes and pectinase are mainly produced by *A. oryzae* and *T. koningii*, whereas SCP is mainly produced by *C. tropicalis*. Therefore, the observed antagonistic effect was probably due to inhibition of the growth of *C. tropicalis* by *A. oryzae* and *T. koningii*.

3.3. Polygalacturonase and carboxymethyl cellulase activity

CMCase and PGase were assessed to determine changes in crude fiber enzymes and pectinase (Wilkins et al., 2007; Solis-Pereira et al., 1993). The activity of both enzymes was expressed as units per mg of SCP feed. As illustrated in Fig. 5 (A), ATP increased as CMCase activity increased when the CMCase activity level was less than 35.26 U/mg, but ATP decreased as CMCase activity increased when the CMCase activity level was greater than 35.26 U/mg. The results shown in Fig. 5 (B) indicated a similar trend, in which ATP decreased as PGase activity increased beyond 33.04 U/mg. These findings suggest that PGase and CMCase inhibit SCP production during fermentation of orange waste.

Moreover, PGase and CMCase facilitated each other during fermentation to increase the degradation rates of pectin and crude fiber. The greatest PD and CFD rates were 19.05% and 22.75%, respectively, when 30.00 U/mg PGase and 35.20 U/mg CMCase were added. These findings are further evidence of the synergistic effect among the 3 tested strains during fermentation of orange waste.

3.4. Colony counting

Colony counting tests were carried out at time-points 1, 2, and 3 to validate the conclusions described above (Fig. 5 C). The quantities of yeast and mold at point 2 were 2.1×10^3 cfu/g and 2.0×10^3 cfu/g greater than those at point 1. The quantity of mold at point 3 was 1.96×10^3 cfu/g greater than that at point 2, confirming the existence of a synergistic effect among the 3 strains.

The quantities of yeast at point 1 and point 2 were only 420 cfu/g and 320 cfu/g less than the quantities of mold, respectively. However, at point 3, the quantity of yeast was 3.7×10^3 cfu/g less than that of mold, and 1.42×10^3 cfu/g less than that at point 2, confirming that the antagonistic effect of the 3 tested strains inhibited

Fig. 4. Synergistic and antagonistic effects of *A. oryzae*, *C. tropicalis* and *T. koningii* during fermentation. (A) The relationship between CFD and PD. (B) The relationship between ATP and PD. (C) The relationship between ATP and CFD. The standard deviations were less than 6% (n = 3), respectively.

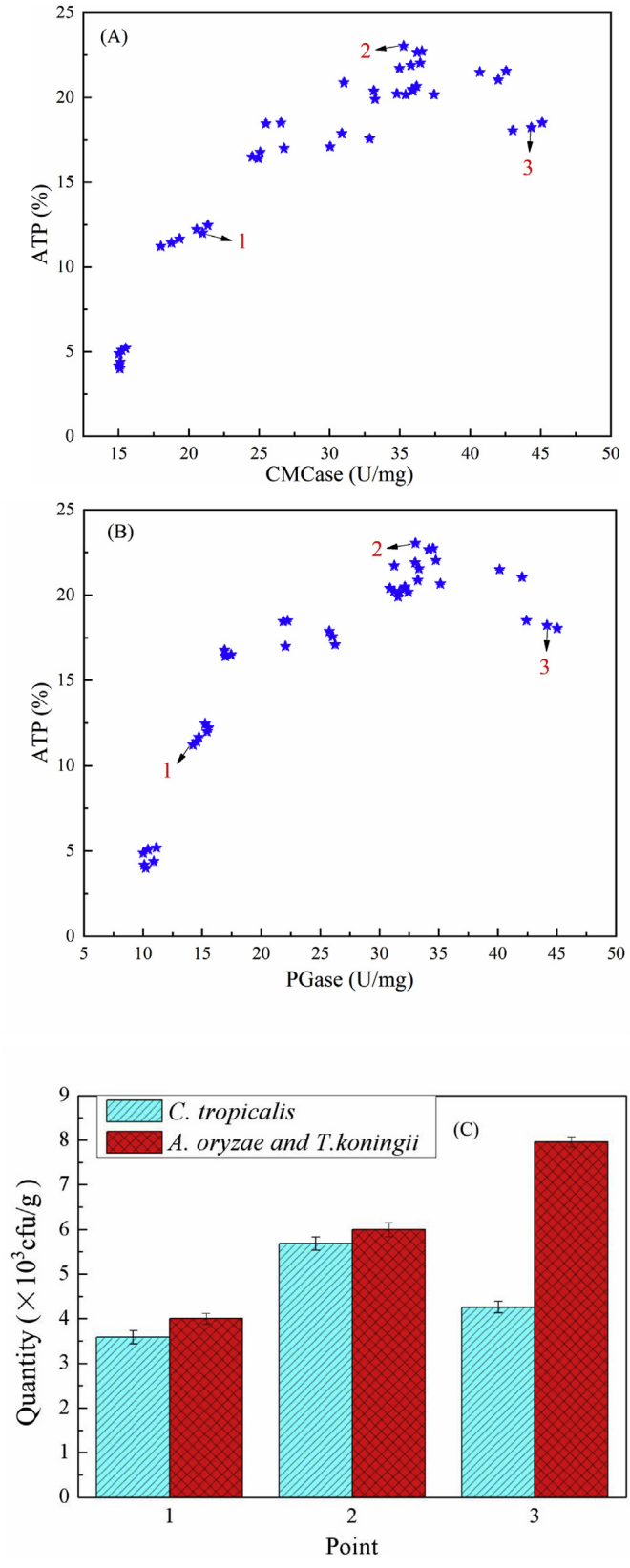


Fig. 5. The validation of synergistic and antagonistic effect by enzyme activity and colony counting. (A) The relationship between ATP and PGase. (B) The relationship between ATP and CMCase. The standard deviations were less than 10% (n = 3), respectively. (C) The quantities of *C. tropicalis*, *A. oryzae* and *T. koningii* at points 1, 2, and 3. Points 1, 2, and 3 represented different relationships among the 3 tested strains. *A. oryzae* and *T. koningii* are molds, and *C. tropicalis* is a yeast.

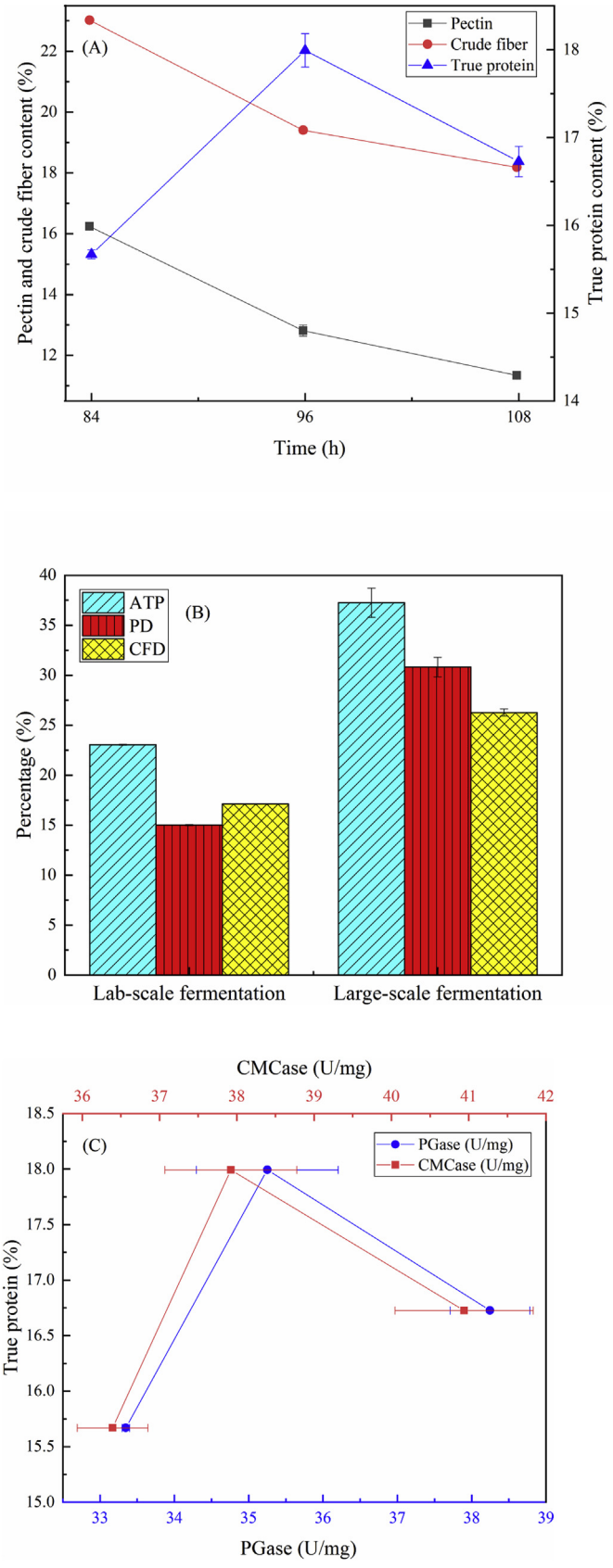


Fig. 6. The SCP feed quality of large-scale fermentation on orange waste. (A) The change of true protein, pectin and crude fiber content over time. (B) The comparison of ATP, PD, CFD by large-scale fermentation and lab-scale fermentation. (C) The relationship between PGase, CMCase and true protein. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Quality of SCP feed produced by large-scale fermentation (% dry weight).

True protein (%)	PGase (U/mg)	CMCase (U/mg)	Pectin (%)	Crude fiber (%)	Ash (%)
17.99	35.25	37.92	12.81	19.41	5.15

the growth of *C. tropicalis*.

Most previous reports have shown only one kind of relationship, either synergistic (Klett et al., 2011) or antagonistic (Lindblom and Tranvik, 2003; Guo et al., 2014), between two strains in a certain environment. However, in reality, both synergistic and antagonistic effects are generally produced, as found in this study.

3.5. Large-scale production of single cell protein feed

The results shown in Fig. 6 illustrate the quality of feed produced in large-scale fermentation and the relationship among the 3 strains tested in this study. The true protein content of the feed gradually increased over time and reached its greatest value of 17.99% at 96 h, after which it decreased. In contrast, pectin and crude fiber content decreased consistently (Fig. 6 A), which was a trend similar to that observed in lab-scale fermentation, and which confirmed the existence of synergistic and antagonistic effects among the strains in the microorganic community. Moreover, the degradation efficiencies of pectin and crude fiber between 84 h and 96 h were 1.54% and 1.15% per hour, respectively, while their degradation efficiencies between 96 h and 108 h were 0.66% and 0.39% per hour, respectively, which demonstrated a significant reduction in degradation efficiency after 96 h. In brief, 96 h was the optimum fermentation time. As shown in Fig. 6 (B), the ATP, PD rate, and CFD rate of large scale fermentation at 96 h were 14.20%, 15.80%, 9.15% greater, respectively, than those observed in the flask test, probably because precise control of DO and agitation speed enhanced microbial activity.

The relationship between PGase, CMCase and true protein is shown in Fig. 6 (C). True protein content increased as PGase and CMCase activity increased when the PGase concentration was lower than 35.25 U/mg and the CMCase concentration was lower than 37.92 U/mg. However, true protein content decreased when the PGase concentration was greater than 35.25 U/mg and the CMCase concentration was greater than 37.92 U/mg, which demonstrated the existence of synergistic and antagonistic effects in the mixed strain community.

The final quality of SCP feed produced by large-scale fermentation is shown in Table 4. The feed was determined to be suitable for chickens (GB/T 5916, 2008) and pigs (GB/T 5915, 2008) in China.

3.6. Economic analysis

The market price of SCP feed in China is dictated by its nutritional value (protein, enzymes, vitamins, etc.). The handling expense for fresh orange waste is 0.063 USD/kg. Approximately 1.2 kg of fresh orange residue (wet weight) can be used to produce 1 kg of SCP feed. The cost of SCP feed produced by solid fermentation of orange waste is 50% of the cost of piglet feed. As shown in Table 5, the total average cost of SCP feed in 2018 is 0.134 USD/kg, whereas a reasonable market price is 0.619 USD/kg, so the net income from SCP feed production is 0.485 USD/kg. The output of orange residue in Changshou Agricultural Park of TGRA is approximately 120 tons annually, which requires the expenditure of 7560 USD on handling expenses. However, this orange waste could be reused as SCP feed to achieve an annual profit of 48500 USD and thus produce significant economic benefits.

Table 5
Expenditure of SCP feed by fermentation of orange waste.

Charge items	Cost (USD/kg)
Auxiliary material	0.028
Labor	0.072
Utilities	0.004
Facility	0.030
Total	0.134

4. Conclusion

Synergistic and antagonistic effects existed simultaneously among the 3 strains during mixed fermentation. Protein production was inhibited when the PGase and CMCase activity levels were higher than certain threshold values. PGase and CMCase are thought to be mainly produced by *A. oryzae* and *T. koningii*, whereas proteins are generally produced by *C. tropicalis*. The 3 tested strains promoted each other when the colony numbers of *A. oryzae* and *T. koningii* were below certain values. However, growth of *C. tropicalis* was inhibited when the colony numbers of *A. oryzae* and *T. koningii* were higher than certain values. Furthermore, large-scale fermentation improved the quality of SCP feed via precise control of DO and agitation speed. The annual profit from reusing orange waste in the Changshou Agricultural Park of the TGRA as SCP feed is 48500 USD, whereas the waste disposal cost of this orange waste is 7560 USD. Therefore, production of SCP by fermentation of orange waste from the TGRA is an effective agricultural residue recycling method.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2018.12.168.

Conflict of interest

The authors declare no conflict of interest.

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