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PRODUCTION OF MICROBIOLOGICAL PROTEIN FEED FROM SWEET POTATO (*Ipomoea batatas* L. Lam) RESIDUE BY CO-CULTIVATION *SACCHAROMYCES CEREVISIAE* AND *CANDIDA UTILIS*

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ABSTRACT

Sweet potato residue (SPR), a byproduct generated during starch extraction from sweet potato, is usually treated as an agro-waste, resulting in lower economic benefits and serious environmental pollution. To promote resource recycling, SPR was used as a starting material to prepare a microbiological protein feed (MPF) by co-cultivating *Saccharomyces cerevisiae* and *Candida utilis*. The cultivation conditions were investigated, and the optimum conditions were determined as follows: inoculation amount of *S. cerevisiae*, 5%; inoculation amount of *C. utilis*, 5%; water content of SPR, 75%; urea, 1%; cellulase, 0.5%; and isoamylase, 0.5%, at a culture temperature of 32°C. Under these conditions, true protein content in fermented SPR was enhanced to 18.08%, which was 6.34 times that of the original SPR. Amino acid composition and the essential amino acid index (EAAI) indicated the high nutritive value of MPF to cattle, rabbits, and lambs. This simple method provides a strategy for recycling and reusing agricultural residues in an economical and environment-friendly manner.

Key words: Sweet potato starch residue, Protein Feed, *Saccharomyces cerevisiae*, *Candida utilis,* Resource regeneration.

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INTRODUCTION

Sweet potato is an important root crop, with a global annual production of more than 1 ×10^8 t (Yang *et al.*, 2017). It plays an important role in food supply, particularly in Asia and Africa (Wu *et al.*, 2018), and is also an essential raw material for various processes (Lai *et al*., 2016). China is the largest cultivator of sweet potato worldwide (Gao *et al*., 2000). In recent years, the proportion of sweet potato processing has gradually increased. Approximately, half (45–50%) of sweet potatoes are used for postharvest manufacturing (Ma *et al*., 2013), and starch products occupy the dominant position since sweet potato is a starch rich crop. It was estimated that approximately 4.5–7.0 tons of fresh sweet potato residue (SPR) is generated during the processing of 1 ton of starch (Arachchige *et al*., 2020; Arachchige *et al*., 2021). Consequently, a large volume of SPRis produced as a byproduct without effective utilization

Additionally, China, as well as other developing countries, is seriously short of protein feed resources (Masuda *et al.*, 2012). In China, animal feed imports increased 49 times between 1980 and the 2010s (Bai *et al.*, 2018), and livestock production in China is increasingly dependent on feed imports. Therefore, it is important to find substitutes for animal feed. Agricultural byproducts, such as maize straw, sweet potato leaves, and SPR, are potential feed sources based on the increasing land occupation for food production. Among these agricultural byproducts, SPR is a promising feed source because of its huge quality and health benefits of fiber and pectin in SPR (Arachchige *et al*., 2020; Liu *et al.* 2020). However, only a small proportion of SPR is used as animal feed, because protein content of SPR is low (~3%, dry basis), while the vast majority of them are directly thrown out and consequently become a burden to the environment (Wang *et al*., 2016).

In recent years, microbiological protein feed (MPF) production using waste products from the food industry or agricultural solid wastes has become the focus of current research and development projects (Gelinas *et al*., 2007; Rajesh *et al*., 2010; Pagana *et al*., 2014), because MPF production has been extended from feed production to environment preservation. If SPR is treated reasonably to enhance the nutritive value, it can be an ideal feed resource and is worth developing.

The protein content is one of the most important quality indicators of MPF. *Saccharomyces cerevisiae* and

Candida utili have been proved to have a high ability to produce protein from several waste substances (Rajoka *et al*., 2004; Khan *et al*., 2010; Hezarjaribi *et al*., 2016). Furthermore, the two yeast strains are both classified as GRAS (Generally Recognized as Safe) microorganisms (Nayak, 2011; Akanni *et al*., 2015). Therefore, in this study, the SPR was used as the starting material to prepare MPF by the cultivation of *S. cerevisiae* and/or *C. utilis,* which has rarely been reported previously. To increase the protein content in SPR, the cultivation technology was optimized, and then the quality of the MPF prepared from SPR was evaluated. This study may provide a novel way to transform agricultural residues into high-value products.

MATERIALS AND METHODS

Materials: SPR was acquired from Jinmei Ltd. (Sichuan, China) immediately after separating from starch and was stored at 4°C.

S. cerevisiae strain CCTCC M206111 was separated from wine lees in Chengdu Institute of Biology, Chinese Academy of Sciences. *C. utilis* CICC 1314 was purchased from the China Center of Industrial Culture Collection, CICC. The yeast strains were maintained on malt wort agar medium at 4°C.

Commercial α-amylase and glucoamylase were purchased from Novozyme (Denmark). α-Amylase had a declared activity of 90 KNU/g glucoamylase had a declared activity of 500 AGU/g, with KNU and AGU defined as follows: 1 KNU (Kilo Novo α-amylase Unit) is defined as the amount of enzyme that can hydrolyze 5.26 g of soluble starch per hour at pH 5.6 and 37°C, while 1 AGU (Amyloglucosidase Novo Unit) is defined as the amount of enzyme which cleaves 1 mmol of maltose per min at pH 4.3 and 25°C (Novo. 1981). Cellulase was purchased from Habio Enzyme, Ltd. (China), with a cleared activity of 1000 U/g. The amount of enzyme required to produce 1 μmol of reducing sugar per minute per gram from the filter paper was defined as 1 U. Pectinase was purchased from Habio enzyme Ltd. (China), with a cleared activity of 1000 U/g. The amount of enzyme required to produce 1 μmol of reducing sugar per minute per gram from the pectic polysaccharide was defined as 1 U. Isoamylase was purchased from Habio enzyme Ltd. (China), with a cleared activity of 5000 U/g. The amount of enzyme required to produce 1 μmol of reducing sugar per minute per gram from the amylopectin was defined as 1 U.

Preparation of the MPF from SPR: The yeast strains were added to 250-ml flasks containing 100 ml of sterilized medium, respectively. The medium composition was as follows: 100 g/L glucose, 1.3 g/L $(NH_4)_2SO_4$, 0.1 g/L $MgSO_4$ and 8.5 g/L yeast extract. Then the culture was incubated at 30°C for 14 h.

The fresh SPR was liquefied using α-amylase (10 min, 85°C, pH 5.2, 120 KNU/kg starch). All cultivations were carried out using a 250-ml beaker containing 100 g of liquefied SPR, urea, cellulase, pectinase and isoamylase (optimized dosage of the supplements is described in the results). Next, 10 ml (OD_{600}=1) of yeast culture was added to the beaker. Glucoamylase was added at a dosage of 750 AGU/kg starch at the time of inoculation. After mixing well, cultivations were carried out at a specific temperature for 3 days.

Experimental design: To investigate the effect of water content (A), temperature (B), inoculation amount (C), and urea addition (D) on the protein production by *S. cerevisiae*, a four-factor and three-level orthogonal test L9 (3^4) was designed. The levels for water content are 65%, 75%, and 85%. The levels for temperature are 25°C, 28°C, and 32°C. The levels for inoculation amount are 5%, 7.5%, and 10%. The levels for urea are 0.5%, 1%, and 1.5%.

To investigate the effects of isoamylase (E), cellulase (F), and pectinase (G) on the protein production, a three-factor and four-level orthogonal test L9 (4^3) was designed. The levels for the enzymes are 0%, 0.25%, 0.50%, and 0.75%.

To investigate the effects of temperature (H), inoculation amount (I), and urea addition (J) on the production of protein by *C. utilis,* a three-factor and two-level orthogonal test L4 (2^3) was designed. The levels for temperature are 28°C and 32°C. The levels for inoculation amount are 5% and 10%. The levels for urea are 1% and 1.5%.

Quality traits of the MPF from SPR: The moisture content was determined by gravimetric analysis after drying a sample at 105°C until a constant weight was achieved. Biomass yield (dry matter content) was determined according to the moisture content of SPR (dry matter content = 100%- moisture content). Starch content was determined by acid hydrolysis and HPLC as described previously (Xiao *et al*., 2013). SPR was hydrolyzed by 1.2M HCl in a boiling water bath for 2h. After adjusting the pH to 7 with 10M NaOH, PbAc was added to precipitate protein. After the solution was filtered and treated with C18 extraction column, the glucose content in hydrolysate was analyzed by HPLC (Thermo 2795, Thermo Corp., USA) with Evaporative Light scattering Detector (All-Tech ELSD 6000, All-tech., Corp., USA). Then the starch content was determined using the total glucose content (starch content = glucose content × 0.909). The true protein content was determined by the method described previously (Marais *et al*., 1983). Protein in SPR was precipitated with trichloroacetic acid to avoid the interference of inorganic nitrogen to the subsequent Kjeldahl Determination. Then the nitrogen in the precipitated protein was determined using a FOSS KJ2200 System (FOSS Corp., Sweden) and multiplying the obtained value by a conventional factor of 6.25. Crude fiber was the loss on ignition of the dried residue remaining after digestion of SPR with 1.25% H_2SO_4 and 1.25% NaOH

solutions according to method No. 32-10 as described by the AACC (American Association for Clinical Chemistry). The ash content was determined gravimetrically in a muffle furnace at 550℃ for 4h (Zhang *et al*., 2011). The optical density (OD) was measured using a 754N UV spectrophotometer (APL Instrument (Shanghai) Co., Ltd.). The amino acid composition was determined by acid hydrolysis and HPLC as previous method (Wu *et al*., 2020), and the detector was set at 440 nm for proline and 570 nm for the other amino acids. The equations for the true protein yield and essential amino acid index (EAAI) were as follows:

True protein yield (g/100g fresh SPR) = biomass yield (g/100g fresh SPR) × true protein content (%, DW)

EAAI = n/{(aa1/AA1) × (aa2/AA2) … (aan/AAn)}

Where, EAAI is the n th root of the essential amino acids in the SPR (aa) to the content of each of those amino acids in the reference tissue (AA) and n is the total number of amino acids evaluated (Kirimi *et al*., 2020).

Statistical analysis: Every experiment was conducted in triplicate, and the data presented represent mean values. DPS software was used to carry out experimental design. Significant differences between samples were evaluated using Tukey's honest significant difference test by SPSS (v19.0).

RESULTS AND DISCUSSION

Composition of SPR*:* The primary chemical composition of the SPR used in this study is presented in Table 1(dry weight, DW). Moisture content of the fresh SPR is 75.14%. It should be noted that the starch content in SPR was 54.6%, which is consistent with previous reports. The content of residual starch in SPR is generally 42.44% and 60.89%, depending on the extraction technology (Mei *et al.*, 2010; Wu *et al.*, 2012; Zhang *et al.*, 2015). If SPR is discarded directly, the starch is the main chemical oxygen demand (COD) source. However, the residual starch can be easily used as a carbon source by certain microorganisms. Crude fiber content was up to 25.42%, which is consistent with the previous reports (Xia *et al.*, 2020). As shown in Fig. 1, there was a large volume of cross-linked cell walls composed of fibers and other constituents. Starch granules were present on or hidden behind the cell wall in sweet potato pulp, whereas the starch granules were mainly wrapped in the piled cell wall in SPR and could hardly be further extracted by traditional starch extraction technology. This phenomenon is consistent with the fact that most of the starch granules in chickpea are still encapsulated by the cell walls after homogenization (Edwards *et al.*, 2021). This is the reason why so much starch is left in the SPR, and the cell wall hampers the further utilization of starch. True protein content was only 2.85% in SPR. A Low protein content and high fiber content result in poor palatability and low nutritional value, which makes SPR unsuitable for direct use as animal feed.

Optimization of the cultivation conditions for *Saccharomyces cerevisiae*: Nitrogen is an essential requirement for microorganism growth. However, the nitrogen content of SPR is low. *S. cerevisiae* are able to use a wide variety of compounds as nitrogen sources (Zhang *et al.*, 2016). To ensure the rapid growth of *S. cerevisiae*, urea was added as an additional nitrogen source for protein synthesis. Thus, during the growth and reproduction of *S. cerevisiae*, cheap inorganic nitrogen sources can be converted into microbial cell protein, which is easily used by animals as MPF. In addition, the water content, temperature, and inoculum size affected the growth of *S. cerevisiae* according to the single-factor tests (data not shown). Therefore, orthogonal experiments with four factors and three levels were designed to screen for optimal conditions for protein yield. As shown in Table 2, the ranges (R) of the inoculation amount (C), water content (A), temperature (B), and urea amount (D) were 1.42, 0.91, 0.79, and 0.32, respectively, indicating that the inoculation amount had the greatest influence on the protein content of SPR, whereas the amount of urea had the least impact. The optimum conditions for microbiological protein yield were an inoculation amount of 10%, water content of 75%, a temperature of 32°C, and 1.5% urea, and under these conditions the protein content increased to 9.46%.

Effect of enzyme addition on protein yield: The low fluidity of the mash for the cultivation of *S. cerevisiae* from SPR resulted in low efficiency of mass and heat transfer. SPR contains abundant cellulose and pectin, which have a strong water-holding capacity (Arachchige, *et al.*, 2020; Xia *et al.*, 2020). Therefore, the viscosity of the mash was very high (Wang, *et al.*, 2016), even when the water content of the SPR was 85%. Moreover, the amylopectin content of sweet potato starch was approximately 75% (Zhang *et al.*, 1999). It was difficult to hydrolyze the α-1,6-glycosidic bonds of amylopectin by glucoamylase (Sidebottom *et al*., 1998). Isoamylase can cleave the branching points of amylopectin to form starch, which can be easily hydrolyzed by glucoamylase (Hill *et al.*, 2012). Therefore, the effects of isoamylase (E), cellulase (F), and pectinase (G) were investigated in this study. According to the single-factor tests (date was not shown), orthogonal experiments with three factors and four levels were designed to screen the optimum conditions for protein production.

The results showed that cellulase significantly enhanced protein content of SPR (Table 3). This may be because cellulase hydrolyzs cellulose to produce glucose and monosaccharides which can be consumed by *S. cerevisiae*. On the other hand, cellulase reduced the viscosity of SPR, which was beneficial for mass and heat transfer (Lai *et al*., 2021). Isoamylase also had a significant influence on protein content, whereas pectinase had only a weak influence. However, in the single-factor test, the

results were opposite. This may be because it was difficult for isoamylase alone to get in contact with starch that was enwrapped by the piled cell wall. After hydrolysis by cellulase, the cell wall was broken, and then isoamylase could more conveniently hydrolyze starch. The optimized comprehensive level combination was E 3 F 3 G 3, but it only had weak superiority over the group E 2 F 2. Considering economic costs into account, the group E 2 F 2 (0.5% isoamylase and cellulase, respectively) was optimal, with which the protein content reached 12.01%.

Optimization of the cultivation conditions for *Candida utilis:* To investigate the effect of cultivation conditions on the production of protein by *C. utilis*, orthogonal experiments were designed. In fresh SPR, the original water content was observed to be about 75%, and the optimum water content for *S. cerevisiae* to produce MPF was also 75%. Therefore, to eliminate the need for water content adjustment, the water content was fixed at 75% in the further experiments based on the above experimental results. Consequently, the addition of enzymes, which is related to the fluidity of the mash, was fixed at 0.5% cellulase and 0.5% isoamylase. Therefore, three factors of temperature (H), inoculum size (I), and urea addition (J) were examined. The range (R) values were 2.05, 1.1, and 0.38, respectively, which indicated that temperature had a greater influence on *C. utilis* than on *S. cerevisiae.* Therefore, the optimum conditions for *C. utilis* cultivation were an inoculation amount of 10%, a temperature of 32°C, and a urea content of 1% (Table 4). That is, the optimized cultivation conditions were almost the same as those determined for *S. cerevisiae*, except for the amount of urea. Under these conditions, the protein content was enhanced to 16.36%, higher than the protein content obtained by *S. cerevisiae* under inoculation amount of 10%, water content of 75%, temperature of 32°C, and 1.5% urea.

Mixed strain cultivation: Different strains had different effects on protein yield. To investigate whether the co-culture of the two strains had a synergistic effect on the enhancement of protein yields, three ratios of the two strains were compared: Ⅰ (*C. utilis*: *S. cerevisiae*=4:1), Ⅱ (*C. utilis*: *S. cerevisiae*=1:1), and Ⅲ (*C. utilis*: *S. cerevisiae*=1:4). Since the optimized cultivation conditions for *C. utilis* were almost the same as those for *S. cerevisiae*, except for the amount of urea, and the effect of urea on protein yield by *S. cerevisiae* was not significant ($p > 0.05$), the dosage of 1% urea was adopted after taking economic considerations into account. Therefore, the experiments were carried out under the optimum conditions as follows: 0.5% cellulase, 0.5% isoamylase, a water content of 75%, a temperature of 32°C, and a total inoculation amount of 10% for the two yeasts, and 1% urea.

As shown in Fig. 2, after cultivation, the protein contents in groups Ⅰ, Ⅱ, and Ⅲ increased dramatically when compared with the original SPR ($p < 0.01$). However, dry biomass yields decreased because of the conversion of carbohydrates in the SPR to volatile matter during the growth and metabolism of the two microorganism strains. The biomass yields of groups Ⅰ, Ⅱ, and Ⅲ were 17.82g, 17.2 6g, and 16.93g per 100g of fresh SPR, respectively. Group Ⅱ (1:1 ratio of the two strains) demonstrated the highest protein content of 18.08% (DW) and the highest protein yield of 3.12g/100g fresh SPR, which were 6.34 times and 4.33 times, respectively, compared to those of the original SPR ($p < 0.01$).

When evaluating different sources of protein, the quantity of essential amino acids is one of the most important indicators of the nutritional value. The essential amino acid index (EAAI) is proposed by Tacon and Cowey (1984) to compare ratios of essential amino acids in a food item to those of a reference pattern derived from whole-body tissue of the animal (Kent *et al.*, 2015). Tissues of lamb, chicken, rabbit, cattle, and pig were selected as reference tissues, since these animals are mostly often raised by farmers. A protein with EAAI greater than or equal to 0.90 is classified as good-quality protein sources, EAAI between 0.80-0.89 is classified as useful protein sources, and EAAI less than 0.70 is classified as low-quality protein sources (Hontiveros *et al.*, 2015). Interestingly, the total amino acids content in group Ⅱ was 24.20%, and the EAAIs were 1.07, 0.97, 1.09, 1.15 and 0.99, respectively, when the meat of lamb, chicken, rabbit, cattle, and pig was used as reference tissues, respectively (Leekim *et al.* 1977; Loëst *et al.*, 1999; Mahan *et al.*, 1998; Zyl *et al.*, 2003) (Table 5). Based on the above-mentioned criteria, fermented SPR could be considered as a source of animal feed with high-quality protein or as an ingredient to improve the amino acid profile of animal feed. Among the reference tissues, fermented SPR had the highest EAAI (1.15) for cattle, whereas fermented SPR had the lowest EAAI (0.97) for chicken. These results indicate that the MPF produced from SPR is more suitable for cattle.

In recent years, several approaches have been developed to solve the problem of SPR by-product accumulation. Bridgers *et al.* (2010) developed enzymatic hydrolysis technology using SPR to generate fermentable sugars. Huang *et al.* (2019) produced succinic acid with engineered *Escherichia coli* strains from SPR hydrolysate. Liu *et al.* (2020) isolated dietary fiber from SPR and proved that dietary fiber is a potential prebiotic food product for improving intestinal health. Arachchige *et al.* (2020) prepared pectin from SPR using an ultrasound/microwave-assisted acid extraction method, and they found that high hydrostatic pressure-assisted pectinase treatment modified pectin with better emulsifying properties. These technologies drastically enhanced the value of SPR. However, after the active ingredients were extracted, the remaining ingredients were still present and acted as secondary pollutants. In addition, sweet potato is often considered a crop grown on small farms (Mukhtar *et al.*,

2010). Sweet potato planting is usually geographically scattered. The planting and processing of sweet potatoes primarily depend on the manual labor of the farmers. During the sweet potato harvest season, most farmers will carry out starch extraction at home using small-scale starch processing equipment, resulting in scattered generated residue. The collection of SPR and its transportation to factories is costly. With the approach developed in this study, farmers can manufacture MPF as soon as starch is extracted from sweet potatoes, and then feed livestock such as cattle on the spot, saving the cost of collection and transportation of SPR. Furthermore, no special equipment or complicated procedures are involved in the production of MPF from SPR by co-cultivating *S. cerevisiae* and *C. utilis*, and the all biomass of the SPR can be recycled without secondary pollution.

Table 1. The primary composition of SPR

Ingredient	Starch	True protein	Crude fiber	Ash
% Dry matter (DM) basis	54.6 ±1.25	2.85±0.04	25.42±0.57	2.67±0.13

Table 2. Effect of cultivation conditions on protein content in SPR by cultivating *Saccharomyces cerevisiae*

Levels*	A/%	B/°C	C/%	D/%	True protein/%
1	65	25	5	0.5	6.38
2	65	28	7.5	1	7.63
3	65	32	10	1.5	8.91
4	75	25	7.5	1.5	8.32
5	75	28	10	0.5	9.23
6	75	32	5	1	8.09
7	85	25	10	1	8.65
8	85	28	5	1.5	8.06
9	85	32	7.5	0.5	8.73
K1	7.64	7.78	7.51	8.11	Primary and secondary
K2	8.55	8.31	8.23	8.12	Factor order: C>A>B>D
K3	8.48	8.57	8.93	8.43	Optimized combination:
R	0.91	0.79	1.42	0.32	A 2 B 3 C 3 D 3

*A: water content; B: temperature; C: inoculation amount; D: urea addition.

Table 3. Effect of enzyme addition on protein content in SPR by cultivating *Saccharomyces cerevisiae*

Levels*	E/%	F/%	G/%	True protein/%
1	0	0	0	9.42
2	0	0.25	0.25	10.66
3	0	0.5	0.5	11.64
4	0	0.75	0.75	11.69
5	0.25	0	0.25	9.77
6	0.25	0.25	0	10.93
7	0.25	0.5	0.75	11.79
8	0.25	0.75	0.5	11.85
9	0.5	0	0.5	10.22
10	0.5	0.25	0.75	11.08
11	0.5	0.5	0	12.01
12	0.5	0.75	0.25	12.13
13	0.75	0	0.75	10.39
14	0.75	0.25	0.5	11.21
15	0.75	0.5	0.25	12.15
16	0.75	0.75	0	12.18
K1	10.85	9.95	11.13	Primary and secondary
K2	11.09	10.97	11.18	Factor order: F >E >G
K3	11.36	11.90	11.23	
K4	11.48	11.96	11.24	Optimized combination: E3 F3 G3
R	0.63	2.01	0.11	

*E:isoamylase; F: cellulase; G: pectinase.

Table 4. Effect of cultivation conditions on protein content in SPR by cultivating *Candida utilis.*

Levels*	H/°C	I/%	J/%	True protein/%
1	28	5	1	13.21
2	32	5	1.5	14.88
3	28	10	1.5	13.93
4	32	10	1	16.36
K1	13.57	14.05	14.79	Primary and secondary
K2	15.62	15.15	14.41	Factor order: H>I>J
R	2.05	1.1	0.38	Optimized combination: H2 I2 J1

* H: temperature; I: inoculation amount; J: urea addition.

Table 5. Essential amino acid composition (% of protein) and essential amino acid index (EAAI) of fermented SPR relative to reference meats.

	SPR	Lamb[a]	Chicken[b]	Rabbit[b]	Cattle[c]	Pig[d]
Arg	4.14	6.94	-	6.19	6.6	6.5
His	2.49	2.61	-	2.70	2.5	3.7
Ile	5.23	3.19	5.54	4.82	2.8	3.9
Leu	7.42	7.19	7.63	8.06	6.7	7.1
Lys	6.46	7.03	8.24	9.15	6.4	7.6
Met	1.98	2.08	2.60	2.62	2.0	1.9
Phe	4.24	4.15	4.15	3.40	3.5	3.8
Thr	5.88	3.79	4.11	4.09	3.9	4.0
Val	5.88	4.28	5.27	4.82	4.0	4.7
Trp	0.92	-	1.06	0.25	-	1.1
EAAI		1.07	0.97	1.09	1.15	0.99

[a] Loëst *et al.*(1999)
[b] Leekim *et al.*(1977)
[b] Zyl *et al.*(2003)
[c] Mahan *et al.*(1998)

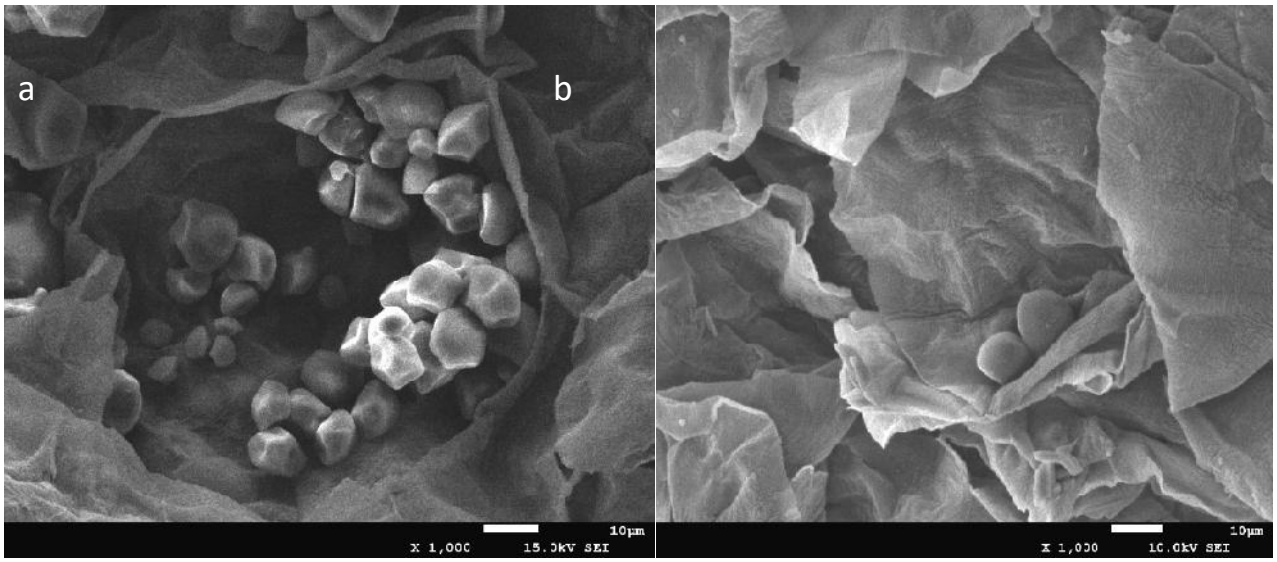

Fig. 1. Scanning electron microscope images of sweet potato pulp and SPR
a: sweet potato pulp (1000×); b: SPR(1000×)

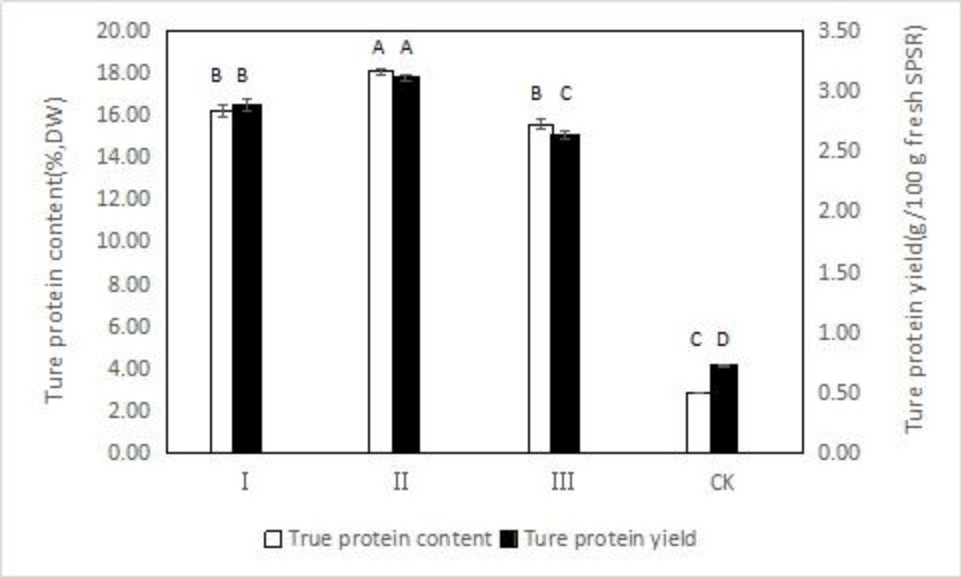

Fig. 2. Effect of different strain mixture ratios on the protein content and protein yield in SPR
Ⅰ: *Candida utilis*: *Saccharomyces cerevisiae*=4:1; Ⅱ: *Candida utilis*: *Saccharomyces cerevisiae*=1:1; Ⅲ: *Candida utilis*: *Saccharomyces cerevisiae*=1:4; CK: the original SPR; Different letters denote significant difference from a Tukey's HSD test ($P < 0.01$)

Conclusions: In this study, the production of MPF from SPR by *S. cerevisiae* and/or *C. utilis* was investigated. The optimum conditions were determined as follows: a 5% inoculation amount of *S. cerevisiae*, a 5% inoculation amount of *C. utilis*, a water content of 75%, a temperature of 32°C, and 1% urea, 0.5% cellulose, and 0.5% isoamylase. Under these conditions, the true protein content in SPR reached 18.08%, which was 6.34 times that of the original SPR. Amino acid composition and the EAAI indicated a high nutritive value of the MPF made from SPR to cattle, rabbits and lambs. Therefore, this study demonstrates a novel bioconversion method to produce high-quality and high-yield protein feed.

Authors' contributions: Yanling Jin: Conceptualization, Methodology, and Writing-original draft. Fan Ding: Resources, and Investigation. Weiliang Shen: Formal analysis, and Data curation. Yang Fang: Project administration. Zhuolin Yi: Writing-review & editing. Lin Yang: Visualization. Hai Zhao: Funding acquisition, Supervision, and Writing-review & editing.

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