

Bioconversion of Apple Pomace into Microbial Protein Feed Based on Extrusion Pretreatment

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Abstract

Apple pomace (AP) is often used directly as animal feed, while the value of feeding is limited by its low protein content. In this study, extrusion pretreatment was performed for AP, and further fermentation was carried out to improve its nutrition value. Strains suitable for extruded apple pomace (EAP) to produce high-quality microbial protein (MP) feed were screened from 12 different strains. Results showed that *Aspergillus niger* 3.324 (*Asn*), *Candida utilis* 1314 (*Cau*), *Geotrichum candidum* 1315 (*Gec*), *Bacillus subtilis* A308 (*Bas1*) and *Lactic acid bacteria* (*Lac*) were screened as the dominant strains, which exhibited higher feeding value. Strong symbiotic effect was observed in fermentation with mixed strains of *Asn*, *Cau*, *Gec*, and *Lac* at the ratio of 1:1:1:1. Compared with AP, the pure protein content in the optimized fermented EAP (FEAP) was increased by 138% accompanying with a pleasant flavor and taste. The nutrition value of FEAP was characterized by amino acid profiles, it found that FEAP was comparable to other commercial proteins with higher contents of histidine, phenylalanine, threonine and valine. Combination of fermentation and extrusion technology significantly enhanced pure protein content and nutritional composition of apple pomace, which was revalorized as a nutritive animal feed that rich in microbial protein.

Introduction

Apple pomace (AP) is a waste by-product with high value-added obtained after the juice or cider by means of pressing, the main constituents of apple pomace are skins, pulp and seeds.(42) AP was often used directly as animal feed, while the feeding value was limited due to its low protein content and digestibility.(43) Reprocessing could be applied to produce high-value compounds from AP instead of using it directly as animal food or discarding as waste.(14) In recent years, varieties of interesting ways were investigated by several researchers such as producing ethanol, phenolic antioxidant, pectin, and enzyme preparation.(35, 3, 10) However, application of these products was limited due to high production cost.(13) Recently, it had been found that AP could be used as an ideal raw material for fermentation of high-value microbial proteins (MP) to increase the added value of AP.(17)

Numerous researches(26, 9) demonstrated that AP was a suitable substrate for solid state fermentation (SSF), its protein content was enriched by cultured strains in SSF. Rodríguez et al(26) found that protein and fat contents of AP were improved after fermented by autochthonous cider yeast strains, which enhanced nutritional and bioactive properties of AP. Devrajan et al(8) showed that a feed of AP using co-culture of *Candida utilis* and *Kloeckera* exhibited better acceptability and digestibility. The fungal protein production was investigated by using AP as a solid substrate for *Rhizopus oligosporus* growth.(4) Those works focused on single or mixed culture of fungi and yeast, while works on co-culture with fungi, yeast, *Bacillus* and *Lactobacillus* were rarely involved. Meanwhile, it was crucial to find mixed strains that suitable for EAP fermentation to produce MP feed.

Several pretreatment techniques were investigated to improve the utilization rate of by-products including acid/alkaline pretreatment, ultrasound and microwave assisted pretreatment.(28, 11) However, several

fermentation inhibitory compounds such as furfural and hydroxymethyl furfurals were generated during acid and microwave pretreatment.(22) Thus, it was extremely important to choose a more suitable preprocessing method.

Extrusion technology emerged as one of the promising methods for processing various materials. It has the advantages of simple process, low energy consumption and so on (46). Extrusion technology was widely used for lignocellulosic materials treatment. The specific surface areas of cellulose were increased by extrusion process, thereby the enzyme accessibility to cellulose was enhanced.(31, 24) Several researchers(16, 7) found that the extrusion process could be used to improve the physicochemical properties of extrudate. Additionally, fermentation inhibitors were not produced during extrusion process. (20) In recent years, extrusion technology had been used in the preparation of raw materials for brewing beer and producing soy sauce.(45, 6) Those results showed that extrusion pretreatment contributed to improve the properties of raw materials, fermentation capacity and product quality. To the best of our knowledge, the application of extrusion technology was rarely reported in the fermentation process of MP production.

The aim of the present work was to screen the optimum mixed strains suitable for EAP fermentation to produce MP feed. The influence of single strain, mixed strains combination and ratio were investigated for EAP fermentation and the nutrition value of FEAP was characterized by amino acid contents.

Materials And Methods

Materials

AP was provided by Kangyuan Biotechnology Co. Ltd (Zibo, China). Peptone, yeast extract, beef extract, agar, and Man Rogosa Sharpe (MRS) medium were obtained from AOBX (Beijing, China). Glucose, K_2HPO_4 , $MgSO_4 \cdot 7H_2O$, $CuSO_4$, K_2SO_4 , H_2SO_4 were obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Distilled water was prepared in the laboratory.

Strains and cultures

The strains used in this study were described in Table 1. *Asn*, *Aso*, *Trh*, *Trl* were stored in Martin Medium Modified agar slant (0.5% peptone, 0.2% yeast extract, 2% glucose, 0.1% K_2HPO_4 , 0.05% $MgSO_4 \cdot 7H_2O$, 1.5% agar) at 4°C and were cultured in Martin Broth Medium Modified at 30°C and 160 rpm for 24h. *Sac*, *Cau*, *Gec* were stored in yeast extract peptone dextrose (YPD) agar slant (1% peptone, 0.5% yeast extract, 2% glucose, 1.5% agar) at 4°C and were cultured in liquid YPD medium at 30°C and 160 rpm for 24h. *Bas*, *Bas1*, *Bas2*, *Bal*, and *Lac* were stored in Luria-Bertani (LB) agar slant (1% peptone, 0.3% beef extract, 0.5% NaCl, 1.5% agar) and Man Rogosa Sharpe (MRS) agar slant at 4°C, respectively. They were separately cultured in liquid LB medium and MRS medium at 37°C and 160 rpm for 24 h.

Table 1
Strains names and isolate numbers used in this study

Strains	Isolate number	Abbreviation	Description
<i>Aspergillus niger</i>	3.324	<i>Asn</i>	YKB ^a isolate
<i>Aspergillus oryzae</i>	/	<i>Aso</i>	SDUT ^b isolate
<i>Trichoderma harzianum</i>	/	<i>Trh</i>	
<i>Trichoderma longibrachiatum</i>	/	<i>Trl</i>	
<i>Saccharomyces cerevisiae</i>	/	<i>Sac</i>	
<i>Candida utilis</i>	1314	<i>Cau</i>	
<i>Geotrichum candidum</i>	1315	<i>Gec</i>	
<i>Bacillus subtilis</i>	3301	<i>Bas</i>	SDUT ^b isolate
<i>Bacillus subtilis</i>	A308	<i>Bas1</i>	
<i>Bacillus subtilis</i>	N52	<i>Bas2</i>	
<i>Bacillus licheniformis</i>	/	<i>Bal</i>	
<i>Lactic acid bacteria</i>	/	<i>Lac</i>	
a Kangyuan Biotechnology Co. Ltd			
b Laboratory of College of Agricultural Engineering and Food Science, Shandong University of Technology			
c China Center of Industrial Culture Collection			

Material pretreatment

AP was pretreated by using single screw extruder (College of Agricultural Engineering and Food Science, Shandong University of Technology). Extrude apple pomace (EAP) was obtained with the extrusion parameters of screw speed 160rpm, sleeve temperature 110°C and material moisture content 26%.

Screening dominant strains for EAP fermentation

In this work, 10% (v/w) strain suspension was inoculated in each 200 mL Erlenmeyer flask containing 45 g sterilized substrate (EAP and bran in 9:1 ratio, blend and water in 1:2 ratio, supplemented with other medium constituents (%w/w): 1% CH₄N₂O, 2% (NH₄)₂SO₄, 0.2% MgSO₄·7H₂O, 0.2% K₂HPO₄) under aseptic condition. Thereafter, those flasks were incubated at 30°C for 4 days.

Screening the optimal mixed strains combination for EAP fermentation

Based on the results of single strain fermentation, *Asn* was ensured to be included in each combination, because of its strongest ability to secrete cellulase among the dominant strains, hydrolyzing a large amount of cellulose in the fermentation substrate. Therefore, the double-strains combinations included *Asn + Cau*, *Asn + Gec*, *Asn + Lac*, *Asn + Bas1*; the three-strains combinations included *Asn + Cau + Gec*, *Asn + Cau + Lac*, *Asn + Cau + Bas1*, *Asn + Gec + Lac*, *Asn + Gec + Bas1*, *Asn + Lac + Bas1*; the four-strains combinations included *Asn + Cau + Gec + Lac*, *Asn + Cau + Gec + Bas1*, *Asn + Cau + Lac + Bas1*, *Asn + Gec + Lac + Bas1*; and the five-strains combination included *Asn + Cau + Gec + Lac + Bas1*.

Screening the optimal mixed strains ratio for EAP fermentation

Based on the test schedule of orthogonal test $L_{16}(2^4)$, the different ratio of *Asn:Cau:Gec:Lac* included 1:1:1:1, 1:1:1:2, 1:1:2:1, 1:1:2:2, 1:2:1:1, 1:2:1:2, 1:2:2:1, 1:2:2:2, 2:1:1:1, 2:1:1:2, 2:1:2:1, 2:1:2:2, 2:2:1:1, 2:2:1:2, 2:2:2:1, marking as group A to O in turn.

Analytical methods

Pure protein and crude protein content were determined by K9860 automatic kjeldahl nitrogen meter (Shandong Haineng Scientific Instrument Co., Ltd, China). The amino acids were determined by A300 automatic amino acid analyzer (MembraPure, Germany).

Cellulase activity of the fermented EAP (FEAP) was analyzed following as: FEAP (2 g) and 90 mL water and 10 mL citric acid buffer were added in 200mL Erlenmeyer flask, which extracted at 40°C for 1h and filtered for later use. Sodium carboxymethyl cellulose solution (Na-CMC, 2 mL) was added in a test tube, which were preheated in a water bath at 50°C for 2 min to 3 min. Then, diluted enzyme solution (0.5mL) was added in this tube, placing in a water bath at 50°C for 30 min. Lastly, 3,5-dinitrosalicylic acid (2.5mL) was added in the tube and boiled for 5min, and the absorption value was measured at 530nm after cooling. According to the following formula (1), the cellulase activity of the sample was calculated, where B – the net glucose content detected from the standard curve (mg), n – the dilution times of enzyme solution, 0.5 – the milliliter number of the diluted enzyme solution absorbed during the determination (mL), t – response time (min), M – molar mass of glucose $M(C_6H_{12}O_6) = 180.2g/mol$. A unit of enzyme activity (U) was the amount of enzyme required to hydrolyze 1 μ mol glucose from 1mg/mL Na-CMC in 1min at 50°C.

$$\text{cellulase activity}(U/g) = \frac{B \times n \times 1000}{0.5 \times t \times M} \quad (1)$$

Effective viable counts of FEAP were analyzed following as: FEAP (1 g) was taken by the quartering method and diluted to different multiples. Several 1:10 (v/v) dilutions were plated for fungi counts in Rose Bengal Medium and for bacterial counts in LB Medium and for *Lac* counts in MRS Medium. In a sterile ultra-clean table, the diluted solution (0.1mL) was taken and cultivated on plates, that were incubated for colony development at 30°C for 48 h.

Statistical analysis

The sample analyses were performed in triplicate. SPSS19.0 was used for the analysis of data and Origin9.0 was used to plot the data. Means were separated using Duncan's comparison at 95% confidence level ($p < 0.05$).

Results And Discussion

Effects of single strain on FEAP

As shown in Fig. 1, the pure protein content of *Asn* (Fig. 1a) was significantly higher ($p < 0.05$) than other fungi strains, indicating that EAP was a suitable substrate for the growth of *Asn* to produce MP. There was no significant difference ($p > 0.05$) in crude protein content between *Asn* and *Aso* (Fig. 1b). The crude protein content of *Asn* and *Aso* were significantly higher ($p < 0.05$) than that of other two fungi strains, suggesting that *Asn* and *Aso* were more suitable to grow on EAP compared with *Trh* and *Trl*. In a similar study, Aruna(5) found that crude protein content during *Trichoderma viride* fermentation of pineapple peels increased from 4.5–14.9% with the addition of $(\text{NH}_4)_2\text{SO}_4$ as nitrogen source. The cellulase activity of *Asn* was significantly higher ($p < 0.05$) than that of all other strains (Fig. 1c), indicating that a large amount of cellulose in EAP was hydrolyzed by the growth of *Asn*. Effective viable count of *Asn* was the highest among fungi strains (Fig. 1d), which was corresponding to its pure protein content. Those results demonstrated that the increase of protein content was caused by the MP produced by the growth of strains.

Cau produced a higher pure protein yield compared with other two yeast strains (Fig. 1a), its crude protein content had no significant difference ($p > 0.05$) with that of *Gec* (Fig. 1b). However, the protein contents of *Cau* and *Gec* were lower than that of *Asn* and *Aso*, indicating that a large amount of cellulose in EAP could not be fully utilized by them due to yeast strains possessed low hydrolytic enzyme activity.(41) Effective viable count of *Cau* and *Gec* were significantly higher ($p < 0.05$) than that of *Sac* (Fig. 1d), suggesting that *Cau* and *Gec* were more suitable to grow in the EAP substance compared with *Sac*. In similar research, Zhu et al (49) reported that *Candida utilis* and *Geotrichum candidum* were the ideal fermentation combination during yellow wine wastes as fermentation substrates. It was worth mentioning that the growth of *Cau* and *Gec* led fermentation products to aromatic flavor, which could improve its palatability.

The pure protein content of *Bas2* was significantly higher ($p < 0.05$) than other bacillus strains, while that of *Bas* was the lowest among all strains (Fig. 1a). Those results indicated that the growth of *bacillus* strains was not conducive to the accumulation of proteins in fermentation products. The main reason might be that the proteins of EAP were hydrolyzed by the proteases produced during the growth of bacillus.(39) *Bas1* and *Bas2* exhibited stronger ability to produce cellulase than *Bas* or *Bal* (Fig. 1c). The effective viable count of *Bas1* was significantly higher ($p < 0.05$) than that of other strains (Fig. 1d), indicating that *Bas1* was more suitable for growing on EAP, and its spore content was higher

correspondingly. Their spores were not easily killed, which were fed into the digestive system of animals as living bacteria.(15)

The pure protein of *Lac* was only higher than that of *Bas* or *Bas1* (Fig. 1a), and its crude protein was only lower than *Asn* or *Aso* (Fig. 1b). It was probably because of its main role in fermentation was not to increase protein content. The cellulose of EAP substrate was not adequately utilized due to the use of *Lac* alone showed low cellulase activity. Therefore, pretreatment or mixed fermentation with other strains were required for *Lac* fermentation. Abdel-Rahman et al (1) found that cellulase pretreatment was helpful for *lactic acid bacteria* to ferment on cellulosic substrates. The effective viable count of *Lac* was lower than that of *Bas1*, *Cau* and *Gec* (Fig. 1d). *Lactic acid bacteria* survived in low pH environments, resisting passage through the small intestine.(27) Furthermore, it showed inhibitory effect on gram-negative bacteria.(18) The fermentation product of *Lac* led to aromatic smells and good palatability, which was consistent with much of researches.(2, 25) Finally, *Asn*, *Cau*, *Gec*, *Bas1*, and *Lac* were screened as the dominant strains for fermenting EAP to produce MP feed.

Effects of mixed strains on FEAP

The pure protein and crude protein content of mixed strains were significantly higher ($p < 0.05$) than that of a single strain (Fig. 2), demonstrating that there was a mutually beneficial symbiotic relationship between the mixed strains.(49) The pure protein contents of double-strain combinations were lower in comparison with three or four-strain combinations (Fig. 2a). The pure protein content of *Asn + Cau* and *Asn + Gec* showed higher levels than that of single strain (Fig. 1a). The pure protein content of *Asn + Cau + Gec* was the highest among the three-strain combination, indicating that the fungi and yeast strains could grow synergistically in the fermentation substrate of EAP. In a similar report, Xiao et al (44) found that the highest protein content of aquatic macrophytes was obtained from the mixed fermentation of *Aspergillus niger* and *Candida utilis*. The four-strain combination of *Asn*, *Cau*, *Gec*, and *Lac* exhibited a significantly higher ($p < 0.05$) pure protein content than all other combinations, indicating that the nutrient of EAP substrate could be fully utilized by that combination. Meanwhile, organic acids and bacteriocins were produced by *Lac*, inhibiting gram-positive bacteria.(18) The fermentation product led to a unique smell, thereby increasing the food intake of the animal. Meanwhile, the crude protein content of the *Asn*, *Cau*, *Gec*, and *Lac* combination was significantly higher ($p < 0.05$) than that of other combinations. The crude protein content of *Asn + Bas1* and *Asn + Lac + Bas1* were lower than that of other combinations (Fig. 2b), showing that *Bas1* was not conducive to the accumulation of MP in the fermentation products. On the one hand, the reason seemed to be that the proteins in the EAP substrate were hydrolyzed by proteases produced during the growth of *Bas1*.(39) On the other hand, a large number of nutrients in the FEAP were utilized by *Bas1*, which inhibited the growth of other strains.

The cellulose in EAP substrate is hydrolyzed to monosaccharides by cellulase for microbial growth and utilization. Cellulase activity could directly reflect the growth of *Asn* and the degree of cellulose hydrolysis in EAP substrate.(36) As shown in Fig. 2c, the cellulase activity of the combination with *Bas1* was relatively higher due to *Bas1* and *Asn* possess the ability to produce cellulase. However, FEAP in combinations of *Lac* showed lower cellulase activity. Romanens(38) found that *lactic acid bacteria*

exhibited an inhibitory effect on filamentous fungi. At the same time, the aroma of FEAP was increased by the addition of *Lac*, which made the animal more receptive for the MP feed. Some researches demonstrated improve the aroma profile of the fermentation product was improved by the co-cultures of yeasts and *lactic acid bacteria*.(34, 12)The effective viable count of *Bas1*in single strain fermentation was higher than that of mixed strains fermentation (Fig. 1d), indicating that antagonistic effects existed between *Bas1* and other strains in the mixed strains fermentation.(40) Thus, *Bas1* was not considered for MP production by mixed strains fermentation EAP. In summary, the combination of *Asn*, *Cau*, *Gec*, and *Lac* was screened as the optimal mixed strains combination for converting EAP into MP feed.

Effects of mixed strains ratio on FEAP

As shown in Fig. 3, group A exhibited the highest pure protein content compared with other groups (Fig. 3a), and its crude protein content was only lower than that of group N (Fig. 3b). Those results indicated that the four kinds of strains could grow cooperatively and accumulate their own MP to increase the pure protein content under the ratio of 1:1:1:1.(29) The increasing crude protein content in FEAP was mainly due to the addition of inorganic nitrogen source. Moreover, fermentable sugars and inorganic nitrogen in the EAP substrate were converted into strains own MP.(5) When the ratio of *Asn:Cau:Gec:Lac* was 1:1:1:1, the pure protein content of FEAP was increased by 138% compared with EAP (4.13%). Consequently, the mixed strains at the ratio of 1:1:1:1 was more suitable for converting EAP into high-quality MP feed.

As shown in Fig. 3c, cellulase activity of group I was significantly higher ($p < 0.05$) than other groups. *Aspergillus niger* played an important role of the substrate hydrolysis,(30) larger *Asn* amount in the inoculum resulted in higher cellulase activity. The cellulase activity of group G was only lower than that of group I, the inoculation amount of *Cau* and *Gec* was higher than others under this mixed strains ratio. That result indicated that *Cau* and *Gec* were beneficial to *Asn* growth, promoting the production of cellulase when their proportion was higher than certain values. However, the growth of protein-producing yeasts was inhibited by the higher *Asn* proportion in the inoculum. In agreement with previous studies, (48) the growth of yeast was inhibited when the colony numbers of fungi was higher than certain values. A balance among the co-culture strains possessing different functions was achieved, which taking the positive effect for producing MP.(47)

As shown in Fig. 3d, the effective viable count of group A was the highest (2.46×10^9 CFU/g) among other all groups, indicating that the four strains (*Asn:Cau:Gec:Lac*) were fermented at the ratio of 1:1:1:1 with excellent synergistic relationship. Effective viable count of group I was only lower than that of group A, suggesting that higher *Asn* proportion in the inoculums was beneficial to increase the effective viable count in the FEAP. However, high proportion of *Asn* caused a musty taste in the fermentation product, which was not conducive to animal feeding.(37)The proportion of *Lac* in Group B, D, F, H, J and N were relatively higher, their effective viable counts were significantly lower ($p < 0.05$) than that of group A. Although the effective viable count of fermentation product was increased by *Lac* in the inoculums (Fig. 2d), a higher proportion of *Lac* had adverse effect in the fermentation process of mixed strains. In

summary, the ratio of *Asn:Cau.Gec.Lac* at 1:1:1:1 was screened as the optimal mixed strains ratio for producing MP feed by fermentation EAP.

Characterization of microbial protein from EAP

As illustrated in Fig. 4, the amino acids except for lysine and histidine in FEAP were higher than those in EAP. The main reason was probably that lysine and methionine in EAP were incorporated by the yeast. (21) The amino acids in FEAP and EAP were higher than those in AP, indicating that the nutrition value was increased by extrusion and fermentation technology.

Composition of amino acid profile of the FEAP and other source proteins were shown in Table 2. All amino acids except for cystine in FEAP exceeded that of ruminants feed. The essential amino acids (EAA) in FEAP were higher than the Food and Agriculture Organization (FAO) standards.(23) The sum of EAA in FEAP was higher compared with the proteins produced by *Lactobacillus acidophilus* or *Aspergillus niger*,(19) which indicated that the MP feeds produced in this study were superior to that of the single strain fermentation. The content of essential amino acids in FEAP conformed to FAO standard and was also compared to other sources of protein, such as soybean meal and fish meal, which had excellent amino acid components as high-quality protein resources for animal feed application.(33) It suggested that FEAP might be an excellent source of protein for animal feeds, especially for non-ruminant animals. For non-ruminant diets, the main limiting amino acids were lysine, methionine, tryptophan and threonine. Although FEAP contained low lysine, methionine and tryptophan, high threonine contents would benefit the use of FEAP as a protein source in non-ruminant feed.(32) Thus, supplementation of low-cost MP into commercial protein could significantly cut down the cost of animal feed.

Table 2
Composition of amino acid profile of the FEAP with that of other proteins

Amino acids	As percentage of total protein (% protein basis)						
	FEAP	<i>Lactobacillus acidophilus</i> ⁴⁷	<i>Aspergillus niger</i> ⁴⁷	Soybean meal ⁴⁸	Fish meal ⁴⁸	Ruminants feed ⁴⁶	FAO ⁴⁶
Alanine	5.08	6.35	3.56	-	-	-	-
Asparticacid	8.57	6.55	5.98	-	-	-	-
Cystine	-	-	-	1.53	1.31	0.74	2.0
Glutamicacid	11.71	9.43	8.07	-	-	-	-
Glycine	4.60	5.45	2.24	-	-	2.43	-
Serine	4.80	3.65	3.22	-	-	-	-
Tyrosine	1.58	2.73	3.54	3.21	3.08	-	-
Proline	2.66	3.66	3.58	-	-	-	-
Arginine	3.92	5.85	2.50	7.38	5.74	-	-
Histidine	3.60	2.33	1.78	2.67	2.36	-	-
Isoleucine	3.14	3.57	3.44	4.94	4.53	2.57	4.2
Leucine	5.69	5.83	5.27	7.80	7.06	3.80	4.8
Lysine	3.30	4.83	4.50	5.53	8.18	3.20	4.2
Methionine	1.55	2.16	1.43	1.41	2.87	0.72	2.2
Phenylalanine	7.28	3.77	3.02	5.26	3.84	2.20	2.8
Threonine	4.55	3.44	4.40	4.03	4.00	1.97	2.8
Valine	6.29	4.53	3.61	5.51	4.87	2.70	4.2
Sum of EAA	39.32	36.31	29.95				
Sum of NEAA	39.00	37.82	30.19				
EAA/NEAA	1.01	0.96	0.99				
FAO: Food and Agriculture Organization							
EAA: Essential amino acid							
NEAA: Nonessential amino acid							

Conclusions

Co-culture of *Asn*, *Cau*, *Gec* and *Lac* at the ratio of 1:1:1:1 on EAP substrate exhibited the optimal microbial protein yield. Pure protein content of 9.81%, crude protein content of 23.52%, cellulase activity of 5.44 U/g and effective viable count of 2.46×10^9 CFU/g in FEAP was achieved using those fermentation conditions. The microbial protein produced by mixed strains showed excellent amino acid profiles, also was comparable to other sources proteins with higher contents of histidine, phenylalanine, threonine and valine. The application of extrusion and fermentation technology could effectively improve the feeding value of apple pomace and promote the effective utilization of apple pomace resources.

Declarations

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Author's Contributions **Zhe Yang:** Participated in the whole experiment process and drafted manuscript. **Lijun Jiang and Min Zhang:** Participated in part of the experimental design and manuscript preparation. **Wenjing Suo, Yuxin Deng and Haijing Zhang:** Participated in part of the experimental design and results analysis. **Chenjie Wang:** Contributed to the guidance of experimental design and ameliorated the manuscript. **Hongjun Li:** Contributed to the guidance of experimental design and ameliorated the manuscript and provided financial support.

Availability of data and material The data and material could be available on request.

Ethics approval This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

Consent to Participate All the authors agreed to participate in the scientific work.

Consent for Publication All the authors agreed to submit the manuscript.

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Figures

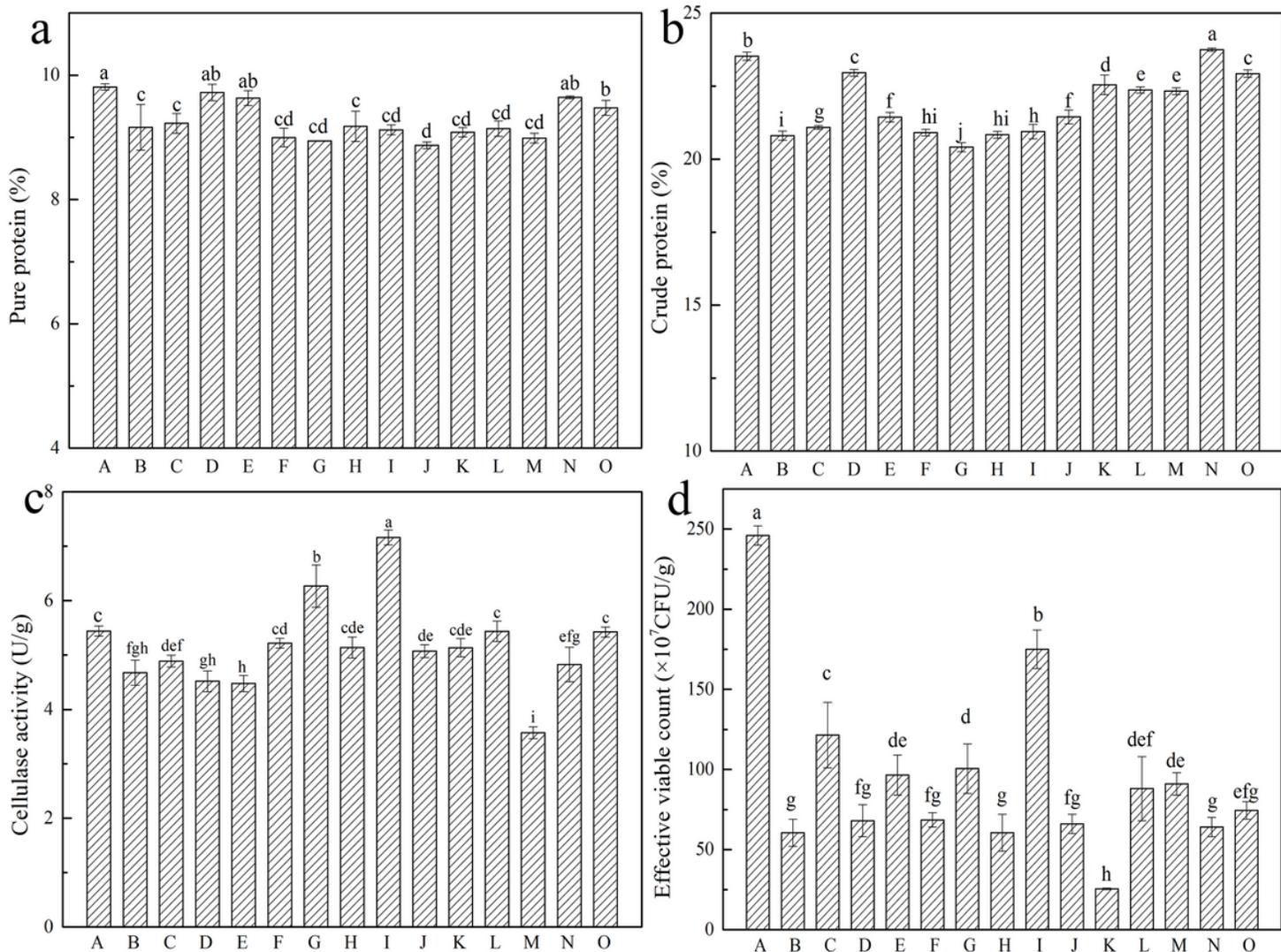


Figure 1

Effects of single strain on pure protein in FEAP (a) Effects of single strain on crude protein in FEAP (b) Effects of single strain on cellulase activity in FEAP (c) Effects of single strain on effective viable count in FEAP (d)

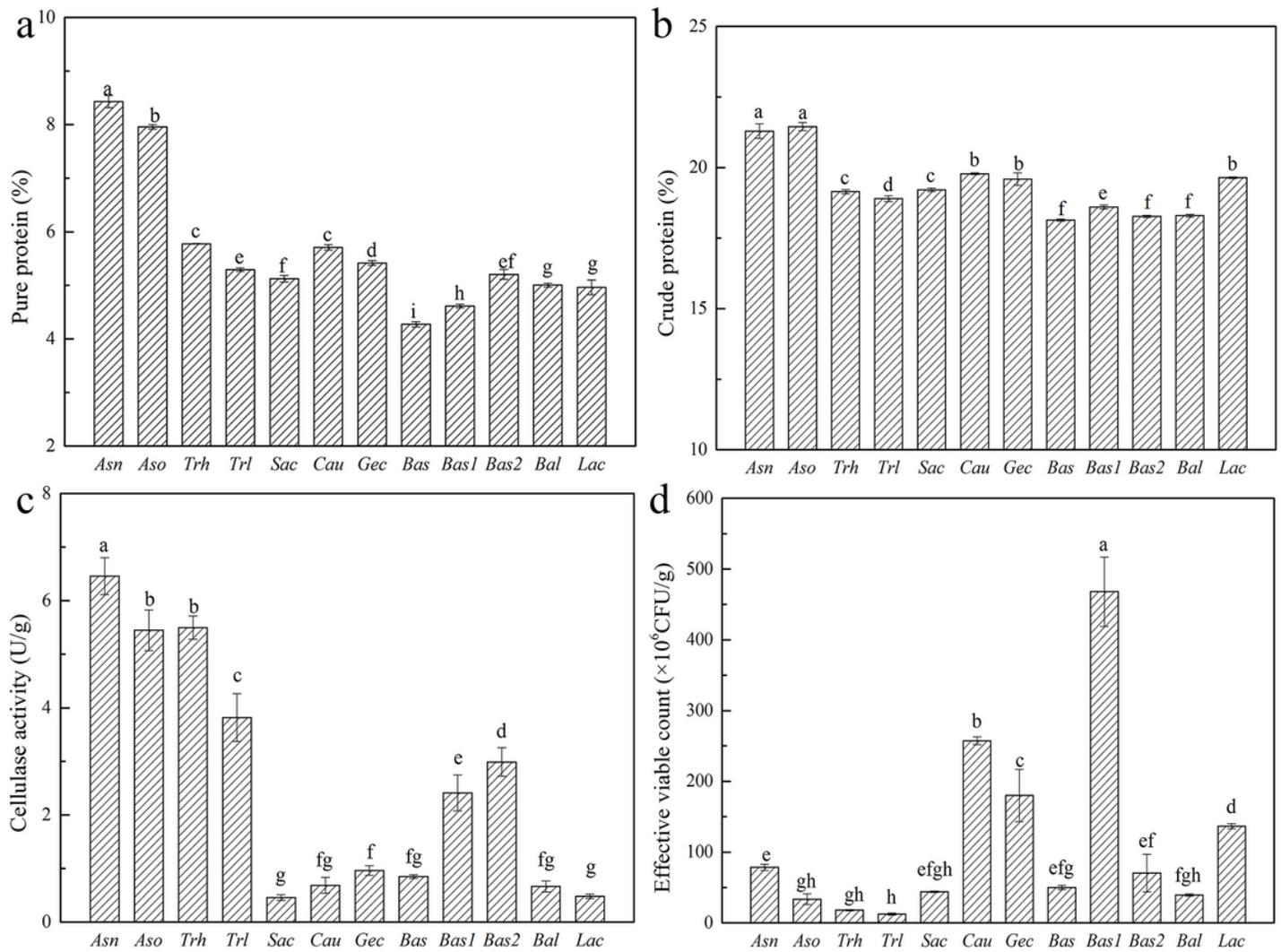


Figure 2

Effects of mixed strains on pure protein in FEAP (a) Effects of mixed strains on crude protein in FEAP (b) Effects of mixed strains on cellulase activity in FEAP(c) Effects of mixed strains on effective viable count in FEAP (d)

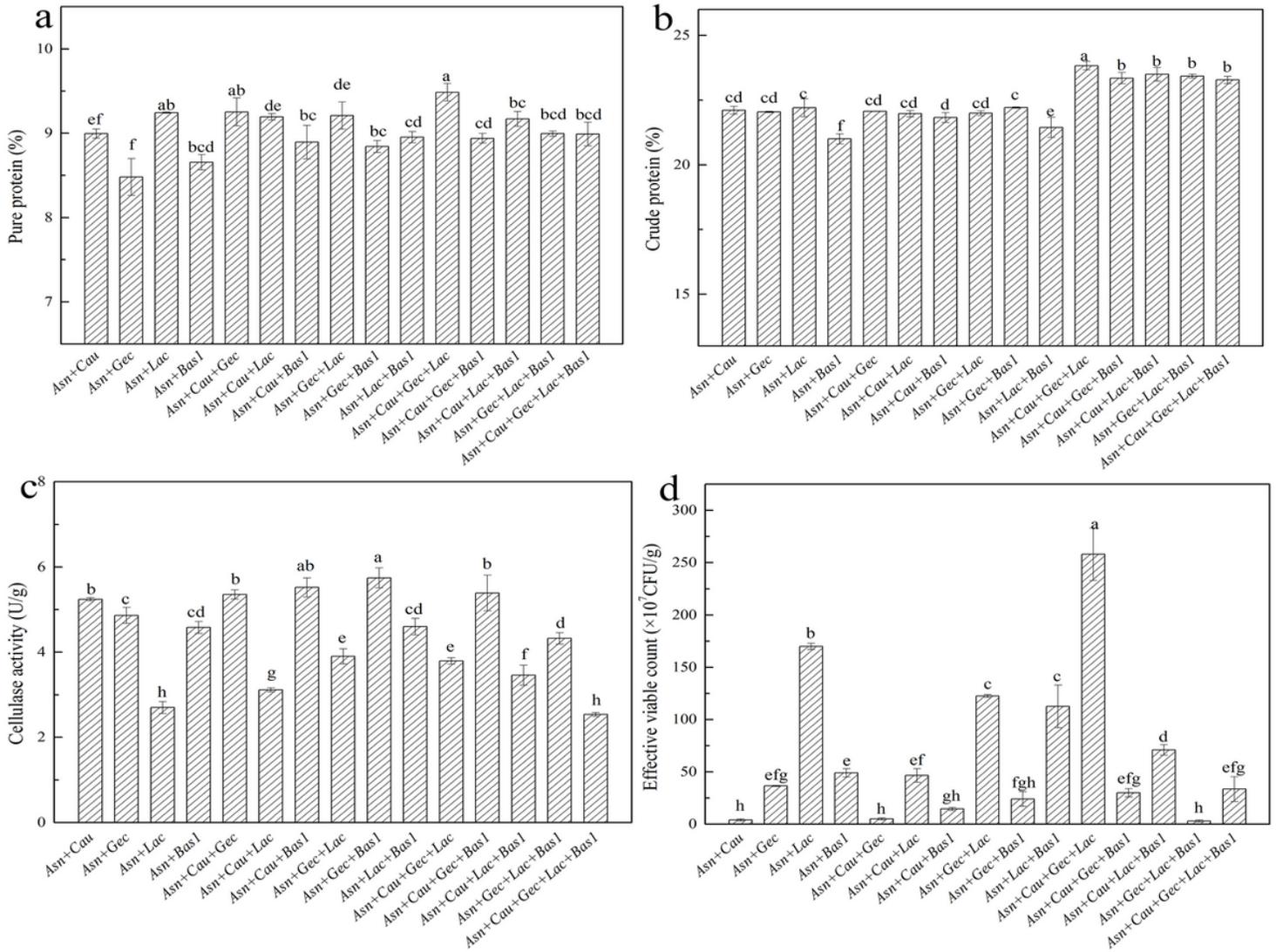


Figure 3

Effects of mixed strains ratio on pure protein in FEAP (a) Effects of mixed strains ratio on crude protein in FEAP (b) Effects of mixed strains ratio on cellulase activity in FEAP (c) Effects of mixed strains ratio on effective viable count in FEAP (d)

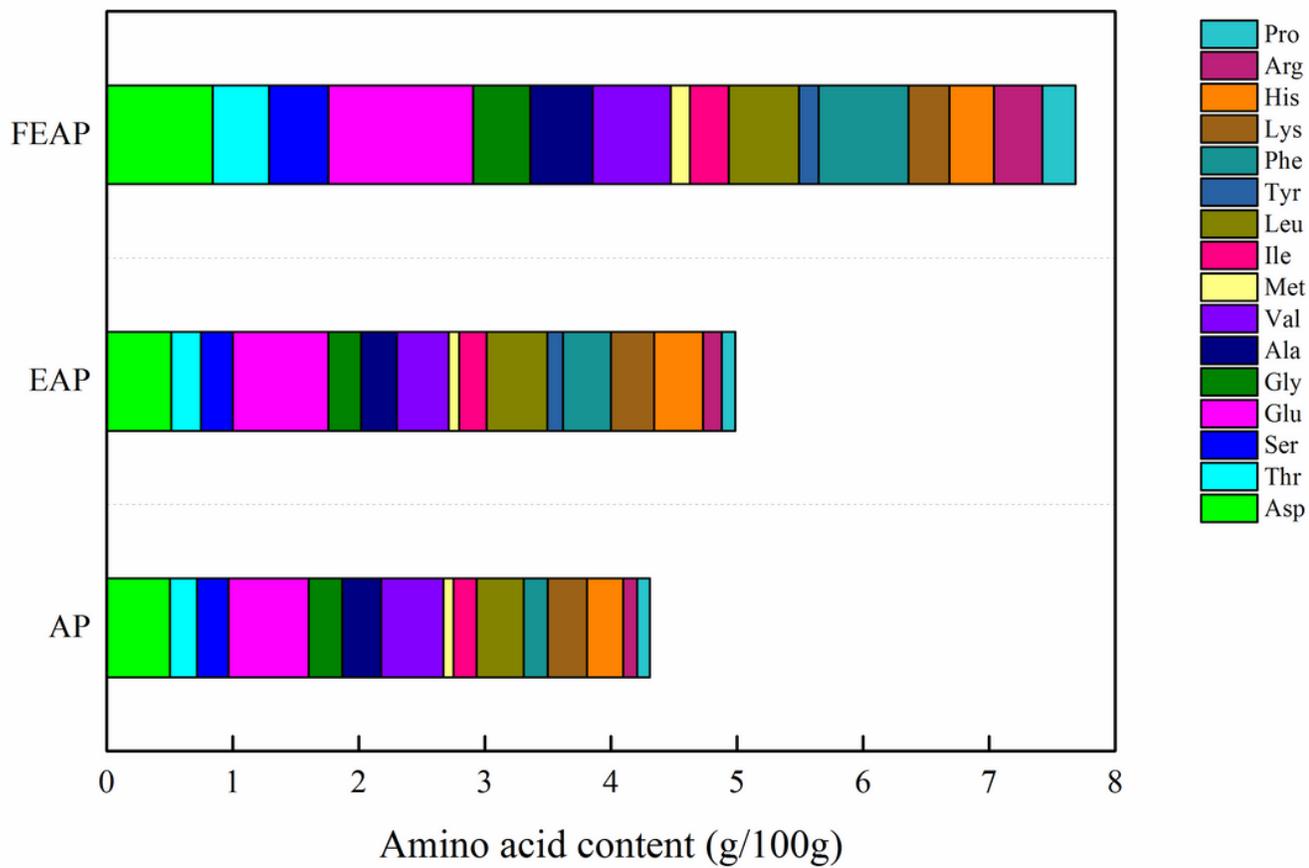


Figure 4

Amino acid contents of AP, EAP and FEAP (g/100g sample)