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Cogent Food & Agriculture (2017), 3: 1295767



Received: 10 August 2016
Accepted: 13 February 2017
First Published: 16 February 2017

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Reviewing editor:
Pedro González-Redondo, University of Seville, Spain

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Proximate and amino acids composition of *Monascus* fermented products with potential as functional feed ingredients

Musaalbakri Abdul Manan^{1*}, Noraini Samat², Madzlan Kasran³ and Hadijah Hassan³

Abstract: Proximate and amino acid composition have been studied from solid state fermentation (SSF) process of broken rice, rice husk pineapple waste and pressed coconut flesh with *Monascus purpureus* FTCC 5391. The main nutrients analyzed after the fermentation are protein, fat, carbohydrate, fiber, ash, and gross energy. *Monascus* fermented products (MFPs) that are known as red fermented broken rice (RFBR), red fermented rice husk (RFRH), red fermented pineapple waste (RFPW) and red fermented pressed coconut flesh (RFCF) have shown increment of protein, fiber and ash while decline of carbohydrate after SSF process. Among all the MFPs, protein content in RFBR (21.22%) had increase 2.3-fold that the highest increment. The calculated gross energy was 456.22, 337.0, 376.76 and 504.4 kcal/100 g for RFBR, RFRH, RFPW and RFCF, respectively. The HPLC analysis indicated the presence of 18 amino acids (Asp, Glu, Ser, Gly, His, Arg, Thr, Ala, Pro, Tyr, Val, Met, Cys, Ile, Leu, Phe, Trp, Lys). Amino acids analysis also indicated that MFPs contained high amount of essential amino acids namely threonine, methionin and lysine that are very important in feed ingredients. These MFPs have a great potential to be used as functional feed ingredients in animal feed formulation.



Musaalbakri Abdul Manan

ABOUT THE AUTHOR

Musaalbakri Abdul Manan is a dynamic, enthusiastic professional and hardworking Research Officer in Biotechnology and Nanotechnology Research Centre. He is a Senior Research Officer in the Enzyme and Fermentation Technology Programme, Biotechnology and Nanotechnology Research Centre, Malaysian Agricultural Research and Development Institute (MARDI), Malaysia. His PhD in Bioprocess was obtained from the School of Chemical Engineering and Analytical Science, The University of Manchester, United Kingdom. He has field experience in microbiology, microbial fermentation and bioprocessing. The research focuses on bioprospecting, preservation and maintenance of microorganisms from local sources in culture collection, utilization of microorganisms through microbial fermentation for the production of value added products, use of process control, recovery process, modeling and simulation for the optimization of fermentation process and evaluation of end-products for safety and quality characteristics. Hence, this study was undertaken to promote the value added products by using of local raw material from agro by-products.

PUBLIC INTEREST STATEMENT

To date, there is no established information of the production of *Monascus* fermented products (MFP) in Malaysia. MARDI has initiated an intensive research on producing MFPs from locally isolated *Monascus purpureus* using solid state fermentation (SSF). The performance of the SSF was influenced by substrate moisture contents and several other factors such as strains of the fungus, substrate variety, nitrogen source and degree of aeration. MFPs which combines the functional characteristics of Monacolin K (anti-hypercholesterolemic agent) is a potential functional bio-ingredients. Its attractive appearance and functional properties can turn MFPs into an important and popular health bio-ingredients in the future.

Subjects: Bioscience; Microbiology; Nutrition; Biotechnology; Agriculture; Food Science & Technology; Food Additives & Ingredients

Keywords: proximate composition; amino acids; *Monascus purpureus*; solid state fermentation *Monascus* fermented products; feed ingredients

1. Introduction

There is a strong need to produce ingredients for new products (food and non-food) from food and agro-industrial residues in a more sustainable way than are currently realized or possible. This is achieved by microbial bioconversions or enzymatic bio-transformations (Koutinas, Wang, & Webb, 2007). Using food and agro-industry solid materials provide almost complete nutrient source (Reed & Nagodawitana, 2008). This is an advantage because during fermentation, solid materials can be used with/without supplementation. Feed ingredients based on microbial bioconversions are still not fully exploited in the animal feed industry (Wang & Pan, 2003). In the case of feed ingredients, research has been developed for its utilization as solid state fermentation (SSF) substrate for added value products. As examples, the production of the antifungal antibiotic iturin (Ohno, Ano, & Shoda, 1992; Takashi et al., 2009), nigerloxin (Chakradhar, Javeed, & Sattur, 2009), meroparamycin (El-Naggar, El-Assar, & Abdul-Gawad, 2009), γ -aminobutyric acid (GABA) and phytic acid (Nagaoka, 2005), lovastatin (Valera et al., 2005), gallic acid (Seth & Chand, 2000), L (+) lactic acid (Naveena, Altaf, Bhadriah, & Reddy, 2005) organic acids (Sauer, Porro, Mattanovich, & Branduardi, 2008), amino acids (Hermann, 2003) and enzymes (Ravindran, 2013) have been reported. Usage will probably increase with the need to have microbial that produce the required feed ingredients but with the zero risk to the animal (Ferrier et al., 1992). Some synthetic feed ingredients have been withdrawn as potential adverse effects on animal health from their use have been identified (Wong, Engku Azhan, & Tan, 2005). The public perception of feed ingredients is that they are safer and better than man-made feed ingredients. Deliberately introduced feed ingredients have become interesting as the manufacture of processed food has grown *Monascus* sp as a source of natural protein and amino acids (Wang, Pan, Shieh, & Hsu, 2006). The ingredients can be extracted and modified to produce bio-ingredients that can be used as feed ingredients. They can also be used directly by growing the mycelium on a solid substrate, which is then dried, grown and incorporated into the animal feed.

SSF and related technologies offer alternative production routes for such biotechnology-based products. SSF has been used in the world for a long time. SSF refers to the microbial fermentation, which takes place in the absence or near absence of free water, thus being close to the natural environment to which the selected microorganisms, especially fungi, are naturally adapted (Musaalbakri, 2014). This technology is commonly known in the East, for traditional manufacture of fermented foods, and in the West, for mould-ripened cheese. It can be defined as a system, in which the growth of selected microorganism(s) occurs on solid materials with low moisture contents and has been identified as a potentially important methodology and technique in biotechnology (Singhania, Patel, Soccol, & Pandey, 2009). One of these fermented products is rice fermented with *Monascus purpureus*. *Monascus*, a traditional Chinese fermentation fungus, has been used in many kinds of foods for thousands of years (Lin, Wang, Lee, & Su, 2008). It is generally recognized for its health benefits because of the proven benefits of the secondary metabolites such as the monacolins (Júzlová, Martínková, & Křen, 1996). Monacolin K is one of the well-documented metabolites of *Monascus* species, which has been identified for their cholesterol-lowering agents due to the competitive inhibitory effect on HMG-Co A reductase (Endo, 1980).

Various agro-industrial residues such as broken rice, rice bran, wheat bran, cassava bran, etc., have been used as substrates by using *Monascus* species especially for pigment production. In this study, broken rice, rice husk, pineapple waste, pressed coconut flesh were defined as waste from different agro-industries, underutilized resource and being primarily used as animal feed. Since the substrates are rich in carbohydrate, protein and trace elements, these can be used as a potential substrate for the production of *Monascus* fermented products (MFPs). Thus, the main objective of this study was to develop fermentation process for the production of pigment employing SSF using

conventional agro-residues and also to study the protein content, amino acid profile, proximate analysis in order to propose them as potential functional feed ingredients.

2. Materials and methods

2.1. The microorganism

The fungus, *M. purpureus* FTC 5391, obtained from the collection of functional food cultures of Biotechnology and Nanotechnology Research Center, Malaysian Agricultural Research and Development Institute (MARDI), Malaysia; was used throughout this study for red pigment production. The strain was improved for its ability in synthesizing red pigment by monospore isolation method. The monospore isolation of red pigment-producing strain was carried out by repeated sub-culturing on solid enrichment medium using spread plate technique and incubation at 32°C for 7 days to obtain progeny at different stages of the subculture. The progeny obtained were sub-cultured on solid enrichment medium and the single colonies or monospores obtained were then transferred to slants for spore formation. Performance of red pigment production by each single colony was compared and the best red pigment producer was selected for further study. Stock culture of the red pigment producer in the form of ascospores, which were attached to porous ceramic beads, was suspended in the cryo-preservative. The culture was placed in a vial and freeze-dried using a laboratory freeze drier according to the standard method. The freeze-dried stock culture in a vial was stored at -80°C prior to use in spore production (Methods by Technical Service Consultants Limited, Europe's Leading Manufacturer of Microbiological Consumables; Mixed Vials of Protected in Polystyrene Tray, Cat. No.: TS70-AS).

M. purpureus FTC 5391, which is a high pigment producer and ascospore-forming, obtained by monospore isolation was maintained on potato dextrose agar (PDA) at 32°C for 7 days. This strain produces compact colonies and accumulates large quantities of red pigments during growth in the form of mycelium.

2.2. Solid-state fermentation

Substrates (broken rice, rice husk, pineapple waste and pressed coconut flesh) were getting from market and factory, were weight (100, 50, 25 and 45 g, respectively) in 500 mL beaker covered with muslin cloth and aluminum foil, and then autoclaved for 20 min at 121°C. Two slants of *M. purpureus* were added to each substrate and 20, 15, 5 and 12 mL distilled water were put into the broken rice, rice husk, pineapple waste and pressed coconut flesh, respectively, and then mixed homogeneously. This amount of water will bring the initial moisture content for each substrate 65%. Each substrate was prepared duplicate. All substrates were incubated in incubator for 7 days at 32°C.

2.3. Proximate composition

Analyses for proximate composition were carried out by AOAC (1990 standard methods). An amount of 5 g samples of the freeze-dried sample was dried for 24 h at 105°C. The loss in weight after drying was recorded as moisture. The ash content of the sample was determined by incinerating a known quantity of the homogenate, previously dried to a constant weight in a silica crucible. The ashing was done in a muffle furnace at 550°C for 24 h until whitish or grayish ash was obtained. The crude fat content was determined by extracting the dried, homogenized sample with petroleum ether using Soxhlet apparatus for about 16 h on an electro thermal extraction unit. Fiber was determined by Weende method using fibertec system (AOAC, 1995 standard methods).

The semi-micro kjeldhal method, which essentially determines the total nitrogen content, was used to determine the crude protein content. An amount of 0.5 g of the homogenized sample was digested with 20 mL nitrogen free concentrated sulphuric acid until the solution become clear. The solution was made alkaline by adding 40% sodium hydroxide solution, steam distilled with 2% boric acid and titrated with 0.02 N sulphuric acid. A factor of 6.25 was used to calculate the protein content. Each analysis was carried out in duplicate. Carbohydrate was calculated by subtracting the

values of moisture, protein, fiber, fat and ash, from 100. Gross energy was analyzed using a bomb calorimeter (C 2000 Basic, IKA® Werke; AOAC, 1990).

2.4. Determination of amino acid composition

2.4.1. Hydrolysis

Individual amino acid were determined after digestion of the samples in 6 N HCl at 110°C as described by Blackburn (1968). The sample was hydrolysed in triplicates by using the sealed-tube hydrolysis method (Davies & Thomas, 1973). About 0.05–0.1 g of the homogenized sample was weighed into a medium wall Pyrex test tube. It was added 10 mL of 6.0 N HCl and the tube was flushed with nitrogen before being hydrolysed in the oven at 110°C for 24 h. After hydrolysis, the sealed end was opened and cooled. The hydrolysate was then transferred to 100 mL volumetric flask and 400 µL of 50 µmole/mL of α -aminobutyric acid (AABA) was added before being made up to volume with deionised water. The hydrolysate was then filtered through filter paper (Whatman No. 541) and re-filtered again with syringe filter (0.45 µm).

Cysteine and methionine were determined by performic acid oxidation prior to their digestion in 6.0 N HCl and were measured as cysteic acid and methionine sulphone, respectively. About 0.05–0.1 g of homogenized sample was weighed into a pear shaped flask. 4.0 mL of cold performic acid was added and the solution was kept in the refrigerator at 4°C for 16 h. 5.0 mL of cold bromic acid was added to the solution and placed in refrigerator for 30 min and evaporated in the rotary evaporator at 80°C. The residue was then hydrolysed with 6.0 N HCl.

Tryptophan was determined by alkaline hydrolysis method with 4.3 N LiOH·H₂O. About 0.05–0.1 g of the homogenized sample was weighed into a medium wall Pyrex test tube. The sample was added with 15 mL of 4.3 N LiOH·H₂O and the solution was flushed with nitrogen to remove soluble oxygen before being hydrolyzed in the oven at 120°C for 16 h. The sample was then transferred into a beaker and added with 9 mL of 1 N HCl. The pH of solution was adjusted to 4.5 with 2.0 N HCl or 2.0 N NaOH. The solution was then transferred to 100 mL volumetric flask and made up to volume with deionized water. The solution was then filtered with filter paper (Whatman No. 2) and the re-filtered with a membrane filter (0.45 µm). 20 µL of the solution was then injected into HPLC column. Each analysis was carried out in duplicate.

2.4.2. Derivatization

Derivatisation was done with borate buffer and ACCQ-tag reagent. 70 µL of borate buffer and 10 µL of sample were mixed and vortex to increased the pH. 20 µL of ACCQ-tag reagent was added and vortex immediately. The sample was kept at room temperature for 1 min for the derivatisation to complete.

2.4.3. Amino acid analysis by HPLC

The amino acid content of the samples was analysed and quantitatively determined using Waters 510 HPLC system (Waters, USA) and detected by Waters 470 fluorescence detector. A 10 µL of derivatised sample was injected into the column and the amino acids were eluted automatically. The quantity of each amino acid was determined from the chromatogram. Mobile phase A was prepared by diluting 200 mL of AccQ Tag Eleunt A (concentrate) with 2,000 mL of distilled water. Mobile phase B was 60% acetonitrile.

2.4.4. Statistical analysis

For qualitative comparison among treatments, proximal composition and amino acid content of MFPs were calculated as the means of two duplicates \pm standard deviation.

3. Results and discussion

3.1. Proximate composition

The nutrient content of solid substrates was influenced by the production of MFPs. For example, broken rice contains high levels of starch (88.8%) (Setyawati et al., 2016) and other major nutrients compared to other solid substrates, which are potential sources for carbon and nutrients for *Monascus* growth.

The proximate composition for each solid substrate tested before and after SSF was summarized in Table 1. In general terms, nutrient composition of fermented products by *M. purpureus* increased compared with before SSF. Protein content increased from 8.98 to 21.22%, 10.76 to 20.0%, 1.09 to 7.16% for broken rice, rice husk and pineapple waste, respectively. Among all the MFPs, protein content in red fermented broken rice (RFBR) had increased 2.3-fold that is the highest increment. Likewise, it showed decreasing protein content in pressed coconut waste (7.3–6.3%), but it was not so high.

The fat content was found slightly increased in RFBR (from 0.41 to 3.27%) and red fermented pineapple waste (RFPW) (from 0.19 to 1.32%) and reduced in red fermented rice husk (RFRH) (from 10.35 to 4.6%) and red fermented coconut flesh (RFCF) (from 64.7 to 30.9%). For carbohydrate and ash content there was not so relevant increase and decrease in all the MFPs. The highest increase in fiber was found in RFPW (from 4.7 to 31.66%) followed by RFBR (from 1.38 to 6.94%). However, the fiber content was reduced in RFRH from 19.61 to 11.82% and no increment in RFCF was observed. In regards to estimated value for gross energy, RFCF had shown promising highest gross energy (504.0 kcal/100 g) after SSF process followed by RFBR (456.22 kcal/100 g), RFPW (376.76 kcal/100 g) and RFRH (337.0 kcal/100 g).

The high protein content of MFPs had shown potential to be developed for wider use and application especially for a good source of protein for feed industry. Research has been done and proven the capability of *Monascus* sp. to produce monacolin K, as a secondary metabolite increased interest of public to use this product as a dietary supplement (Yang & Mousa, 2012). Monacolin K can lower the cholesterol level in body, improving food digestion and blood circulation (Klimek, Wang, & Ogunkanmi, 2009). Consumption of red fermented rice (also known as red yeast rice or red mold rice) has been extensively studied in animals and humans and was found to reduce cholesterol levels by 11–32% and triacylglycerol concentrations by 12–19% (Heber et al., 1999). The effect of red yeast rice supplementation to reduce egg cholesterol level has also been studied and reported by Nuraini and Latif (2012), Wong, Engku Azhan, and Tan (2006) and Wang and Pan (2003). The study showed that red yeast rice supplementation could be used in layer diets to reduce egg cholesterol content with no adverse effects on egg production or feed efficiency. Nuraini and Latif (2012) reported that poultry diet added with 30% fermented products by *M. purpureus* improved performance and reduced egg cholesterol 31.49% and increased yolk colour 18.56%.

Table 1. Proximate analysis of MFPs before and after fermentation process

Nutrient	Broken rice		Rice husk		Pineapple waste		Pressed coconut flesh	
	Before (%)	After (%)	Before (%)	After (%)	Before (%)	After (%)	Before (%)	After (%)
Protein	8.98 ± 0.02	21.22 ± 0.03	10.76 ± 0.05	20.0 ± 0.03	1.09 ± 0.02	7.16 ± 0.03	7.3 ± 0.09	6.3 ± 0.02
Fat	0.41 ± 0.03	3.27 ± 0.04	10.35 ± 0.01	4.6 ± 0.02	0.19 ± 0.07	1.32 ± 0.09	64.7 ± 0.08	30.9 ± 0.17
Moisture content	10.42 ± 0.01	7.30 ± 0.02	8.26 ± 0.04	10.4 ± 0.08	7.9 ± 0.08	3.7 ± 0.01	4.8 ± 0.01	10.2 ± 0.02
Carbohydrate	79.68 ± 0.015	67.37 ± 0.012	52.29 ± 0.02	54.0 ± 0.02	90.45 ± 0.04	84.06 ± 0.12	21.2 ± 0.09	50.1 ± 0.21
Fiber	1.38 ± 0.02	6.94 ± 0.08	19.61 ± 0.03	11.82 ± 0.04	4.7 ± 0.06	31.66 ± 0.15	12.65 ± 0.01	12.71 ± 0.17
Ash	0.51 ± 0.01	0.84 ± 0.03	11.34 ± 0.03	11.0 ± 0.01	0.37 ± 0.01	3.76 ± 0.03	2.0 ± 0.03	2.5 ± 0.03
Gross energy (kcal/100 g)	358.49	456.22	438.43	337.0	367.86	376.76	696.0	504.0

Note: Each figure represents the means of two duplicates ± standard deviation.

3.2. Amino acid composition

The amounts of each amino acid are shown in Table 2. HPLC analysis showed that the precipitate contained 18 amino acids namely aspartic acid (Asp), glutamic acid (Glu), serin (Ser), glycine (Gly), histidine (His), arginine (Arg), threonin (Thr), alanine (Ala), proline (Pro), tyrosin (Tyr), valine (Val), methionine (Met), cysteine (Cys), isoleusin (Ile), leusin (Leu), phenylalanine (Phe), tryptophan (Trp) and lysine (Lys). The most abundant amino acids were Glu followed by Asp and Leu per 100 g sample in all four MFPs. The data also indicated that MFP contained high amount of essential amino acids such as Thr, Met and Lys, which is very important and used abundantly in feed ingredients. RFRH had showed the highest amount of these three essential amino acids compared to other three MFPs as shown in Table 2.

The protein adequacy for a human life depends on the content of essential amino acids (Leu, Ile, Lys, Met, Cys, Phe, Thr, Val, Trp and His). In general, essential amino acids represent 43.72% of the total amino acid. This is an attractive value in terms of nutritive quality. Amount of amino acid in RFBR, RFRH and RFPW shown in Table 2 are 2.53, 16.32 and 1.12% respectively. Even though the amino acids from the three samples showed lower percentage essential amino acids but still can be used in large amount as feed ingredient. This will be very useful as the alternative of the essential amino acid sources. Moreover, results showed RFRH can be a very promising source of animal feed ingredient as highest amount of essential amino acids obtained (16.32%). It is considered that fermentation using *M. purpureus* FTCC 5391 could improve the nutritive protein fractions of solid substrates. However, there may be need for essential amino acid supplementation when such MFPs are used in formulating feed for poultry and livestock animals.

SSF is an environmentally friendly process that consume less energy and produce less waste and can be applied to a variety of different products. The SSF with fungus significantly improves nutritive

Table 2. Amino acid composition (mg/100 g) in *Monascus* based products

Amino acid	RF broken rice	RF rice husk	RF pineapple waste	RF pressed coconut flesh
Asp	215.0 ± 0.01	180.7 ± 0.01	116.0 ± 0.01	31.1 ± 0.09
Glu	474.0 ± 0.01	199.7 ± 0.02	187.1 ± 0.09	64.1 ± 0.11
Ser	150.0 ± 0.03	83.3 ± 0.03	72.1 ± 0.02	18.0 ± 0.02
Gly	175.0 ± 0.09	84.9 ± 0.05	80.0 ± 0.08	17.2 ± 0.07
His	38.0 ± 0.02	21.8 ± 0.07	18.2 ± 0.03	ND
Arg	162.0 ± 0.01	90.4 ± 0.03	48.0 ± 0.07	27.4 ± 0.15
Thr	120.0 ± 0.02	65.1 ± 0.02	57.3 ± 0.05	11.5 ± 0.06
Ala	176.0 ± 0.06	97.9 ± 0.08	79.2 ± 0.06	17.4 ± 0.17
Pro	179.0 ± 0.04	77.1 ± 0.06	59.1 ± 0.01	9.8 ± 0.04
Tyr	143.0 ± 0.09	62.3 ± 0.1	61.1 ± 0.19	10.8 ± 0.03
Val	140.0 ± 0.1	89.4 ± 0.11	70.2 ± 0.04	23.9 ± 0.06
Met	47.0 ± 0.05	30.9 ± 0.01	16.9 ± 0.05	4.9 ± 0.15
Cys	75.3 ± 0.05	52.0 ± 0.01	42.3 ± 0.2	32.7 ± 0.12
Ile	111.0 ± 0.02	63.3 ± 0.18	51.7 ± 0.21	8.9 ± 0.5
Leu	229.0 ± 0.06	117.7 ± 0.12	101.0 ± 0.03	28.5 ± 0.04
Phe	134.0 ± 0.07	74.9 ± 0.2	56.2 ± 0.01	18.4 ± 0.02
Trp	43.2 ± 0.1	39.0 ± 0.01	27.1 ± 0.02	20.3 ± 0.01
Lys	37.0 ± 0.02	40.4 ± 0.12	48.4 ± 0.09	8.9 ± 0.11
Sub-total	2,642.5	1,470.8	1,188.4	353.8

Notes: Each figure represents the means of two duplicates ± standard deviation.

RF—Red fermented; ND—Not determined.

value. This provides a promising future for sustainable industry. In this case, SSF using *M. purpureus* FTC 5391 can support the manufactures to replace the expensive meal to certain levels and support in reducing the feed ingredients cost and thereby increasing the profitability of animal feed system. Nutritive value and amino acid profile were increased by SSF of mentioned solid substrates. MFPs are potential as an alternative protein rich or functional feed ingredient in diet of targeted animal. However, the bioactive components of functional MFPs such as monacolin K and polysaccharides are also important in *Monascus* production.

4. Conclusion

The improvements of nutrient content in MFPs are very potential to be used as feed ingredients. Highest increment of protein in MFPs will give a great nutritive value. A total of 18 amino acids were identified in *Monascus* based products. The most abundant amino acid in RFBR, RFRH and RFPW was glutamic acid. The highest essential amino acid needed in feed ingredient was found in RFRH. RFRH is very potential source for animal feed ingredient, which has the essential amino acids content, was 16.32%. To achieve a balanced nutritional composition in animal feed, a the formulation should be carried out in selecting feed ingredients.

5. Recommendations

Products derived from *Monascus* sp have been shown that can be important ingredients for chicken feed. The finding in the present study suggest that MFPs could be used as protein substitute up to 5–10% in the diet of growing chicken (specific for layers). At the same time, it will carry some benefits on cholesterol properties; for example targeting low cholesterol eggs. However, further studies are needed to justify the long-term effect and benefit of MFPs products for health and egg production. Overall, qualitatively, SSF using *M. purpureus* FTC 5391 have improved the nutrient of MFPs. Further, the quality control and management during the fermentation process must be taken into account. With this adaptation, MFPs is likely to be an appropriate choice for protein supplement and could be scaled up for large production.

Funding

This work was funded by Agro-Biotechnology Institute (ABI) research [grant number RB2090AB10].

Competing Interest

The authors declare no competing interest.

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Citation information

Cite this article as: Proximate and amino acids composition of *Monascus* fermented products with potential as

functional feed ingredients, Musaalbakri Abdul Manan, Noraini Samat, Madzlan Kasran & Hadijah Hassan, *Cogent Food & Agriculture* (2017), 3: 1295767.

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