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Nutritional assessment of *mycomeat* produced from different agricultural substrates using wild and mutant strains from *Pleurotus sajor-caju* during solid state fermentation

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ABSTRACT

Fermented foods have good nutritional and health benefits, and are produced via solid state fermentation (SSF) technology. This study was carried out to produce a feed variety; *mycomeat* through the solid state fermentation using various agricultural by products for livestock feeding. *Pleurotus sajor caju* was cultured on different agricultural substrates at $27 \pm 2\,^{\circ}C$. The mutant strain was produced using random mutagenesis by exposing the wild strain to ultra violet radiation for 30 min. The moisture content of the different agricultural by products was maintained at 60 mg/g and apportioned into three sets: Treatment 1 contained agricultural substrate by product alone. Treatment: 2, in addition to agricultural by product, contained mutant strain of mushroom exposed to ultra violet radiation for 30 min. Treatment: 3, in addition to agricultural by product, contained wild strain of the mushroom. The set-up was incubated in the dark and monitored daily until full ramification was obtained. Strain improvement enhanced dry matter content of *mycomeat* produced from palm kernel meal (82.81 mg/g), the protein content and fat content followed a similar pattern. Strain improvement significantly ($P = 0.1$) reduced the NDF and ADF contents of *mycomeat* produced from palm kernel meal, while fungal treatment reduced the crude fiber content of *mycomeat* produced from palm kernel meal. *Mycomeat* produced by corn bran and palm kernel meal were adjudged the best.

1. Introduction

Agricultural by products are generated in large tons in developing countries and their disposal is currently a major economic and ecological challenge. In some parts of Nigeria, these agricultural by products are allowed to accumulate in large quantities, allowed to decay or are burnt indiscriminately thereby impacting negatively on the environment by releasing harmful volatile compounds into the atmosphere, water and soil and as a result poses a serious threat to human health.

Mushroom is a general term used mainly for the fruiting body of macrofungi (Ascomycota and Basidiomycota) and represents only a short reproductive stage in their life cycle (Das, 2010). Fermented foods are known for their good nutritional

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and health benefits, and are often produced via solid state fermentation (SSF) technology, a process in which micro-organisms are grown on solid substrates in the absence of free water (Lagemaat and Pyle, 2001). Chang and Miles (1989), coined "mycomeat" to refer to fungal protein obtained through the conversion of food processing biomass by products; most times via SSF. Edible fungi, mainly mushrooms can be cultured for their fruiting body, metabolites such as enzyme, or *mycomeat* (containing both the growth substrate and the mycelial of the fermenting fungi). Okwulehie and Ogoke (2013) reported that mushrooms are good sources of proximate components and minerals needed for good health. Mushrooms are valuable health foods that are low in calories, high in vegetable proteins, chitin, iron, zinc, fiber, essential amino acids, vitamins, and minerals, such as copper that help the body to produce red blood cells (Aina et al., 2012). Mushrooms are often regarded as highly nutritious and having many health benefits (Kumari et al., 2011).

Mushroom is an excellent source of essential amino acids, vitamins, and minerals and can contribute to the formulation of a balanced diet (Manzi et al., 2001; Mattila et al., 2001). Therapeutic properties of mushrooms include enhancement of macrophage function and host resistance to many bacterial, viral, fungal, and parasitic infections; activation of a non–specific immune stimulation; and reduction of blood cholesterol and blood glucose levels (Cheung et al., 2003; Rajarathnam et al., 1998). Mushroom has been found to inhibit aromatase activity and suppress breast cancer cell proliferation (Grube et al., 2001).

For like a decade now, it has been discovered than farmers especially those involve in animal production spend more money on the purchase of animal feed which in turn has an adverse effect on their income. Mushroom is an attractive crop to cultivate in developing countries for many reasons. One of the advantages of mushrooms is that they readily grow on agricultural by products. The use of these substrate materials is both cost effective and environmentally friendly. Further, such substrates are readily available. It enables us to acquire substrate materials at low prices or even for free and to conserve our environment by recycling by products. These by products are produced in big tons during the production of agricultural products every year causing lots of environmental problems in many countries (Belewu and Banjo, 2000). Only a very small part of these agro-wastes have been properly converted into useful or high-value products. Production of edible or medicinal mushroom is a successful example of agro-waste recycling (Chiu et al., 2000).

This research work attempts the production of a feed variety; *mycomeat*, through solid state fermentation using various agricultural by products for livestock feeding.

2. Material and methods

Selected agricultural solid by products were sourced from Omu-Aran Community in Kwara State, Nigeria. *P. sajor caju* LMU 01 were procured from the culture collection bank, Department of Microbiology, NIHORT, Ibadan, Nigeria. The cultures were sub-cultured periodically after every 4 weeks and incubated at $25 \pm 1\,^{\circ}C$ for 10 days on Potato dextrose agar (PDA) slants and stored at $4\,^{\circ}C$.

2.1. Inoculums development

The inoculum was developed by transferring loopful of inoculum into the prepared inoculum medium (0.2 mg/g yeast extract, 1 mg/g feather substrate, pH 6.0). 25 mL of the isolate was dispensed in a 150 mL capacity bottle. Incubation was carried out at ($30 \pm 1\,^{\circ}C$) on a thermostatic shaking water bath at 100 rpm for 24 h.

2.2. Exposure of isolated fungus to UV light to induce mutation

This was carried out by preparation of fresh PDA plate to grow the organisms. After the growth of the organisms, cork borer was used to obtain several mycelia plugs from the culture into a sterile PDA plate. The sterile plate containing several mycelia plugs was placed under UV lamp at 300 nm wavelength at a distance of 30 cm to the plates. At time interval of 30 min, 5 mycelia plugs were withdrawn and used as inoculants for scale fermentation studies. The mycelia plugs from the domesticated type culture served as the control (Adetunji and Oloke, 2013).

2.3. Substrate treatments

The by products were prepared according to the method of Akintunde and Akintunde (2002) with a little modification. The moisture content of the different agricultural by products was maintained at 60 mg/g and apportioned into three sets. Treatment 1 contained agricultural substrate by product alone. Treatment 2, in addition to agricultural by product, contained mutant strain of mushroom exposed to UV 30 min. Treatment 3, in addition to agricultural by product, contained wild stain of the mushroom. They were filled into the wide-mouthed transparent jars in triplicates, corked with cotton wool and sterilized in the autoclave at $121\,^{\circ}C$ for 15 min.

2.4. Experimental set-up

The sterilized substrates were inoculated with the mycelia of the edible mushroom; a slant was washed per jar. The set-up was incubated in the dark and monitored daily until full ramification was obtained. The set- up that produced full

Table 1
Interactive effects of agricultural substrates and processing type on chemical composition of *mycomeat* on dry matter basis.

Treatment	Type	Dry matter (mg/g)	Protein (mg/g)	Ash (mg/g)	NDF (mg/g)	ADF (mg/g)	Fiber (mg/g)	Fat (mg/g)	Ca (mg/g)	P (mg/g)	CHO (mg/g)
Wheat bran	Control	85.78^{c}	7.36^{e}	4.09^{e}	21.81^{p}	6.25^{p}	5.12^{p}	4.09^{f}	0.28^{ab}	0.35^{a}	65.12^{d}
	Wild	86.20^{abc}	6.55^{fg}	3.16^{jk}	24.66^{n}	8.26^{k}	5.54^{o}	3.87^{g}	0.27^{ab}	0.23^{bc}	67.08^{b}
	Mutant	86. 07^{abc}	8.11^{d}	3.75^{f}	24.80^{n}	8.50^{k}	7.62^{n}	3.74^{g}	0.27^{ab}	0.33^{ab}	62.88^{j}
Groundnut husk meal	Control	83.81^{e}	6.38^{ghi}	3.68^{fg}	51.15^{d}	27.91e	44.48^{e}	2.17^{l}	0.44^{ab}	0.05^{e}	27.10^{q}
	Wild	83.74^{e}	5.67^{k}	3.41^{h}	50.48^{f}	27.19^{f}	45.15^{d}	2.26^{l}	0.33^{ab}	0.05^{e}	27.25^{q}
	Mutant	83.92^{e}	6.22^{j}	3.48^{gh}	50.89^{e}	27.55^{e}	44.99^{d}	2.34^{kl}	0.33^{ab}	0.05^{e}	30.37^{p}
Moringa oleifera seed husk	Control	86.23^{abc}	9.19^{b}	2.97^{klm}	54.07^{a}	29.62^{b}	50.64^{a}	2.45^{k}	0.34^{ab}	0.07^{de}	20.98^{s}
	Wild	86.13^{abc}	9.25^{b}	2.91^{lm}	53.32^{b}	28.96^{c}	50.33^{b}	2.50^{k}	0.34^{ab}	0.07^{de}	21.14^{s}
	Mutant	85.85^{c}	8.57^{c}	2.79^{m}	53.13^{c}	28.86^{c}	49.95^{c}	2.49^{k}	0.33^{ab}	0.06^{e}	22.05^{r}
Corn bran	Control	86.33^{abc}	10.33^{a}	4.39^{d}	18.88^{r}	4.41^{p}	1.27st	5.13^{b}	0.30^{ab}	0.32^{ab}	65.21^{d}
	Wild	86.15^{abc}	10.29^{a}	4.35^{d}	20.74^{q}	5.88°	1.97r	4.96^{c}	0.30^{ab}	0.32^{ab}	64.58^{f}
	mm	85.96^{bc}	10.35^{a}	4.58^{d}	23.06^{o}	7.65^{o}	2.28^{q}	4.80^{cd}	0.31^{ab}	0.31^{ab}	63.95^{g}
Corn cob	Control	85.74^{c}	1.41^{q}	3.61^{fgh}	50.88^{e}	28.33d	39.64h	1.63^{m}	0.30^{ab}	0.07^{de}	39.45^{m}
	Wild	86.78^{a}	2.34^{o}	3.38^{hi}	54.25^{a}	31.64a	41.03^{g}	1.49^{m}	0.28^{ab}	0.05^{e}	38.54^{n}
	Mutant	86.66^{ab}	1.97^{p}	3.06^{kl}	54.09^{a}	31.55^{a}	42.16^{f}	1.50^{m}	0.26^{b}	0.03^{e}	37.97^{o}
Palm kernel meal	Control	81.00^{f}	4.07^{n}	6.24^{a}	28.33^{j}	10.32^{k}	1.50^{s}	4.73^{d}	0.35^{ab}	0.40^{a}	70.70^{a}
	Wild	80.78^{f}	3.99^{n}	6.04^{a}	24.73^{n}	7.86^{m}	1.20^{t}	4.41^{e}	0.35^{ab}	0.42^{a}	65.15^{d}
	Mutant	82.81^{e}	5.03^{m}	5.13^{c}	18.01^{s}	2.34^{q}	1.01^{t}	5.74^{a}	0.32^{ab}	0.39^{a}	65.90^{c}
Rice bran	Control	84.03^{e}	5.64^{k}	6.09^{a}	50.12^{g}	27.29^{f}	30.02^{j}	2.89^{j}	0.37^{ab}	0.19^{c}	39.40^{m}
	Wild	83.94^{e}	5.57^{k}	5.50^{b}	49.27^{h}	26.28^{g}	29.77^{j}	2.98^{j}	0.37^{ab}	0.19^{c}	40.12^{l}
	Mutant	83.56^{e}	5.32^{l}	5.57^{b}	50.03^{g}	27.03^{f}	10.02^{m}	2.87^{j}	0.37^{ab}	0.18^{cd}	59.78^{k}
Cassava peel	Control	86.70^{a}	6.33^{ghi}	1.51^{n}	26.54^{m}	10.95^{j}	10.82^{l}	3.18^{h}	0.21^{b}	0.18^{cd}	64.86^{e}
	Wild	86.58^{ab}	6.46^{fgh}	1.60^{n}	27.43^{k}	11.43^{i}	10.66^{l}	3.21^{h}	0.22^{b}	0.17^{cd}	64.65^{f}
	Mutant	86.43^{abc}	6.66^{f}	1.54^{n}	26.76^{l}	10.30^{k}	11.44^{k}	3.17^{h}	0.21^{b}	0.17^{cd}	63.61^{h}
	P value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.063	0.001	0.001
	SEM	0.36	0.52	0.28	2.97	2.20	4.03	0.25	0.01	0.03	3.67

Means with different superscripts within the same column are significantly (P = 0.05) different. NDF = neutral detergent fiber, ADF = acid detergent fiber, SEM = standard error of mean, *P* = 0.1.

Table 2
Interactive effect of agricultural substrates and processing type on amino acid profile of *mycomeat* on dry matter basis.

Treatments	Type	Arginine (mg/g)	Cysteine (mg/g)	Isoleucine (mg/g)	Leucine (mg/g)	Lysine (mg/g)	Methionine (mg/g)	Threonine (mg/g)	Tryptophan (mg/g)	Valine (mg/g)
Wheat bran	Control	3.48^{cde}	2.01^{g}	2.53^{f}	4.22^{c}	2.58^{h}	0.00^{h}	2.17^{h}	0.23^{bcd}	3.28^{de}
	Wild	2.87^{fg}	1.70^{h}	1.80^{h}	3.68^{e}	3.33ef	0.28^{e}	1.82^{i}	0.17^{fg}	2.87^{i}
	Mutant	3.77^{c}	1.67^{h}	2.20^{g}	3.68^{e}	2.86^{g}	0.00^{h}	1.87^{i}	0.20^{def}	2.87^{i}
Ground nut husk meal	Control	3.52^{cde}	3.07^{d}	2.27^{fg}	3.03^{g}	2.36^{i}	0.00^{h}	2.56^{cde}	0.03^{kl}	2.92^{ghi}
	Wild	3.54^{cde}	3.07^{d}	2.24^{fg}	3.07^{g}	2.37^{hi}	0.00^{h}	2.59^{cd}	0.03^{kl}	2.92^{ghi}
	Mutant	3.63^{cd}	3.36^{d}	2.50^{f}	3.68^{e}	2.00^{j}	0.00^{h}	2.70^{c}	0.06^{k}	3.45^{d}
*Moringa oleifera*seed husk	Control	4.34^{ab}	3.89^{b}	3.54^{c}	5.75^{b}	1.22^{l}	0.00^{h}	3.35^{ab}	0.22^{bcde}	4.84^{b}
	Wild	4.27^{ab}	3.93^{b}	3.51^{c}	5.69^{b}	1.28^{l}	0.00^{h}	3.37a^{b}	0.20^{def}	4.82^{b}
	Mutant	4.50^{ab}	4.18^{a}	3.89^{b}	6.19^{a}	0.94^{l}	0.00^{h}	3.53^{a}	0.24b^{c}	5.24^{a}
Corn bran	Control	2.81^{fg}	1.27^{j}	1.51^{i}	2.09^{h}	3.74^{c}	0.37^{b}	1.27^{j}	0.12^{hij}	1.73^{k}
	Wild	2.82^{fg}	1.43^{i}	1.56^{hi}	2.19^{h}	3.69^{cd}	0.33^{d}	1.33^{j}	0.11^{ij}	1.89^{k}
	Mutant	2.78^{fg}	1.30^{ij}	1.47^{i}	2.06^{h}	3.75^{c}	0.35^{c}	1.27^{j}	0.13^{ghij}	1.76^{k}
Corn cob	Control	2.60^{g}	1.58^{h}	1.60^{hi}	1.59^{i}	2.51^{hi}	0.00^{h}	1.44^{j}	0.00^{l}	1.33^{lm}
	Wild	4.00^{bc}	2.44^{f}	3.22^{d}	4.19c	1.63k	0.00^{h}	2.49^{cdef}	0.22^{bcde}	3.36^{de}
	Mutant	4.75^{a}	2.97^{d}	4.23^{a}	5.75^{b}	1.00^{l}	0.00^{h}	3.20^{b}	0.36^{a}	4.47^{c}
Palm kernel meal	Control	3.15^{def}	2.07^{g}	2.33^{fg}	3.30^{f}	4.15^{a}	0.00^{h}	1.77^{i}	0.21^{cde}	2.44^{j}
	Wild	3.52^{cde}	2.39^{f}	2.79^{e}	4.23^{c}	3.48^{de}	0.00^{h}	2.21^{gh}	0.25^{b}	3.19^{ef}
	Mutant	3.00^{efg}	2.35^{f}	2.33^{fg}	4.03^{cd}	3.85^{bc}	0.23^{f}	2.18^{gh}	0.23^{bcd}	3.08^{fg}
Rice bran	Control	3.52^{cde}	2.66^{e}	2.32^{fg}	3.25^{fg}	3.17^{f}	0.00^{h}	2.31^{fgh}	0.14^{ghi}	2.84^{i}
	Wild	3.65^{cd}	2.78^{e}	2.49^{f}	3.87^{de}	2.94^{g}	0.00^{h}	2.41^{defg}	0.15^{gh}	3.06^{fgh}
	Mutant	2.58^{g}	0.93^{l}	1.07^{j}	1.26^{j}	4.03^{ab}	0.46^{a}	0.82^{k}	0.11^{ij}	1.19^{m}
Cassava peel meal	Control	3.53^{cde}	2.74^{e}	2.36^{fg}	3.32^{f}	3.19^{f}	0.00^{h}	2.34^{efgh}	0.13^{ghij}	2.89^{hi}
	Wild	2.59^{g}	1.07^{kl}	1.22^{j}	1.62^{i}	3.90^{bc}	0.46^{a}	0.96^{k}	0.10^{j}	1.45^{l}
	Mutant	3.15^{def}	1.18^{jk}	1.74^{hi}	2.17^{h}	3.68^{cd}	0.09^{g}	1.25^{j}	0.19^{ef}	1.81^{k}
	P value	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
	SEM	0.13	0.19	0.17	0.28	0.21	0.03	0.16	0.02	0.22

Means with different superscripts within the same column are significantly (P = 0.1) different. SEM =standard error of mean, P = 0.1.

ramification within the shortest time frame (best mycomeat) was then selected. The fermentation process was done by simple submerge method.

2.5. Design of the study

The design of the study was a 3 × 8 factorial arrangement in a completely randomised design. There were three processing types: control, wild and mutant strain of *Pleurotus sajor caju* while there were eight agricultural by products.

2.6. Proximate and amino acid analyses

The combination of the fungi and substrates were analysed for chemical composition and amino acids. Chemical composition and amino acid profile of selected agricultural solid by products were carried out using DA 7250 NIR analyzer (Pertem, Sweden). Each treatment was done in triplicates. The mean values obtained for proximate composition and amino acid profile were subjected to an analysis of variance using SPSS. Significant means were separated using Duncan's multiple range test.

3. Results and discussion

The colony grew moderately and covered the entire plate within 6 d with white flat cottony surface and dense marginal hypae. Growth of hypae near the inoculums with arachnoids zones and was identified as *Pleurotus cajor saju.*

The interactive effect of agricultural by products and processing types on chemical composition and amino acid profile of *mycomeat* are shown in Tables 1 and 2. The protein content was significantly (P = 0.1) higher for *mycomeat produced* from corn bran. Strain improvement enhanced protein content of *mycomeat* produced from wheat bran. Strain improvement also enhanced protein content of *mycomeat* produced from groundnut seed husk meal (6.22 mg/g) than the wild (5.67 mg/g), while strain improvement enhanced carbohydrate content (30.37 mg/g) than the wild (27.25 mg/g) and the control (27.10 mg/g). Strain improvement reduced fiber content of the *mycomeat* produced from *Moringa oleifera* seed husk meal (49.95 mg/g) than the wild (50.33 mg/g) and the control (50.64 mg/g). Fungal treatment improved the dry matter content of *mycomeat* produced from corn cob, while the protein content followed a similar trend. Strain improvement enhanced dry matter content of *mycomeat* produced from palm kernel meal (82.81 mg/g), the protein content and fat content followed a similar pattern. Strain improvement significantly (P = 0.1) reduced the NDF and ADF contents of *mycomeat* produced from palm kernel meal, while fungal treatment reduced the crude fiber content of *mycomeat* produced from palm kernel meal. Strain improvement reduced fiber content of *mycomeat* produced from rice bran. Strain improvement enhanced cystein, isoleucine and leucine contents of mycomeat produced from *Moringa oleifera* seed husk meal. Strain improvement enhanced arginine content (4.75 mg/g), cystein content (2.97 mg/g), isoleucine content (4.23 mg/g), leucine content (5.75 mg/g), threonine content (3.20 mg/g) and tryprophan content (0.36 mg/g) of *mycomeat* produced from corn cob. Fungal treatment enhanced cystein content, leucine content and threonine content of *mycomeat* produced from palm kernel meal, while strain improvement enhanced its methionine content (0.23 mg/g). Strain improvement also enhanced lysine content (4.03 mg/g) and methionine content (0.46 mg/g) of *mycomeat* produced from rice bran, while fungal treatment enhanced lysine content of *mycomeat* produced from cassava peel meal.

Mushrooms are generally regarded as highly nutritious with accompanied medicinal benefits (Kumari et al., 2011). It has been observed that several lignocellulosic substrates can support the growth of mushrooms (Isikhuemhen and Nerud, 1999). Apetorgbor et al. (2013) earlier reported the range of proximate composition (g/100 g) of *Pleurotus tuber-regium* grown on plantain leaves, water hyacinth, millet stalk and 'wawa' sawdust thus: 10.50 ± 0.03–13.07± 0.08 (ash), 15.92 ± 0.06–40.01 ± 0.15 (crude protein), 14.45 ± 0.04–20.43 ± 0.04 (lignin) and 22.68 ± 0.10–34.04 ± 0.09 (cellulose). Edible mushrooms have been observed as good alternatives to animal protein among rural dwellers who cannot afford the high cost of meat. Mushrooms have been reported to be rich in sugar, amino acids, glycogen, lipid, ascorbic acid, protein, chitin, iron, zinc, fiber, copper and essential amino acids (Kumari et al., 2011; Aina et al., 2012).

Many of the substrates supported the growth of *Pleurotus sajor caju* aside *Moringa oleifera* seed husk meal and cassava peel meal. Moringa have been previously reported to contain anti-microbial properties while cassava is rich in hydro cyanide (Tewe, 1994; Ola-Fadunsin and Ademola, 2014), which could have been responsible for the growth inhibition. The growth period was around 8 or 10 days, which was shorter than the previous findings by Lau et al. (2013) and Bamigboye et al. (2013). The previous work on *mycomeat* production used different mushroom specie, soymilk substrates and it was produced as protein substitute for human consumption, while the present study attempted focused on production of *mycomeat* from different agricultural by products for livestock feeding.

Disposal of agricultural by products resulting in agricultural pollution and economic loss in Nigeria and other developing countries can be profitably controlled by converting them to feed, particularly for monogastric animals. A previous study by Belewu et al. (2004) revealed a higher crude protein content for diet treated with *Pleurotus sajor caju* fed to rat. The improved amino acid reported in the present study can therefore be attributed to the addition of fungal protein synthesized by *Pleurotus sajor caju* (Jacqueline and Visser, 1996). Low content of fiber had also been reported for *Pleurotus sajor caju*-treated rice bran (Belewu et al., 2004). The low content of crude protein, amino acids and other nutrients in agricultural by products, which limit the usefulness of some them as animal feed can be enhanced through fungal (*Pleurotus sajor caju)* treatment (Belewu

et al., 2004). Livestock feeding systems based on fungal treatment seems to be a great opportunity for more effective use of agricultural by products for improved animal production and productivity.

Strain improvement in the field of microbiology is done with the aim of improving the microbes' productivity: to change unused co-metabolites, to improve the use of carbon and nitrogen sources and to improve the morphology of cells in order to separate the cells and its products. Mutagenesis is a process of mutation in strain improvement of microorganism. A treatment to microorganism enhances their genotypic and phenotypic performances. Mutation can be random or direct and may be performed by physical, chemical or combination of physical and chemical method. Ultra violet radiation (UV- light) has been reported as one of the best physical methods of strain improvement for better yield performance (Kang et al., 1999; Oloke et al., 2012). This method has been employed in improving enzyme production in *Aspergillus niger, Rhizopus oryzea* (Soden et al., 2002) mycelia cell and sporophore production in *Pleurotus florida* and *Pleurotus sajor-caju* (Riva, 2006).

4. Conclusion

The proximate analyses and amino acid profile of the feed variety (*mycomeat)* revealed rich nutritional content which may be explored for feed ingredient in livestock production. It is concluded that the findings did not only support the bioremediation of agricultural by products to produce high-value bio-product but also provide evidence that improvement of microorganism strain represent a viable way to enhance nutritional value of fermented products.

Conflict of interest

Authors declare that no conflict interest exists concerning this manuscript.

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