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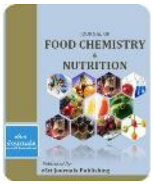

CHARACTERIZATION OF NUTRIENTS IN CAROTENOID-ENRICHED FULL FAT SOY FLOUR AND RICE BRAN PRODUCED BY RED YEAST FERMENTATION

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ABSTRACT

Carotenoids are used as feed additives for improved animal health and to produce quality animal products. Fermentation of corn whole stillage, rice bran, soybean flour etc by carotenogenic or red yeasts can result in carotenoid-enriched animal feed. Usually, the prescribed dietary dosage of carotenoids like astaxanthin and β-carotene is 1-120μg/g feed. In a previous study, *Phaffia rhodozyma*-fermented full fat rice bran and *Sporobolomyces roseus*-fermented full fat soy flour resulted in highest astaxanthin yield of 80μg/g feed and β-carotene of 836μg/g feed respectively. The aim of this study was to qualitatively and/or quantitatively evaluate the nutrition profiles of these two fermented products. In both products, there was reduction in crude fiber, crude protein and amino acids whereas the crude fat content was enhanced in rice bran and reduced in soy flour respectively; the levels of some amino acids like hydroxyproline, hydroxylysine and ornithine were enhanced; oleic acid increased by 83% in *S. rosues*-fermented soy flour whereas stearic acid was enhanced by 389% in *P. rhodozyma*-fermented rice bran; and trypsin inhibitor was reduced to undetectable levels in carotenoid-enriched soy flour. Both fermented substrates contained 1 to 3% glucans and, 2.4 to 5.7% mannans, and glucosamine ranging from 0.13 to 4.6%. In addition to high levels of carotenoids, red yeast fermentation of both soy flour and rice bran yielded a suite of nutrients and the final product could be used to make 'feed blends' to provide adequate nutrients based on the dietary requirements of the animals.

Keywords: Glucan, glucosamine, mannan, *Phaffia rhodozyma, Sporobolomyces roseus*.

Abbreviations: ADF Acid Detergent Fiber. ATCC American Type Culture Collection. DDGS. distillers dried grain with soluble. K Potassium. MOS Mannan Oligosaccharide. NDF Neutral Detergent Fiber. N Nitrogen. P Phosphorous. S sulfur. TI Trypsin inhibitor.

INTRODUCTION

Yeast and yeast-based products that are typically rich in proteins, vitamins and minerals have been used as animal feed additives and are known to promote animal health resulting in improved quality of animal products. Supplementation of active dried brewer's yeast *Saccharomyces cerevisiae* in various animal feeds is known to improve immune response, confer disease resistance and offer protection from pathogens (Bontempo *et al.,* 2006; Burgents *et al.,* 2004; Li and Galtin III 2005), improve milk yield in dairy cattle (Desnoyers *et al.,* 2009; Robinson and Erasmus 2009; Wang *et al.,* 2009) and enhance animal growth and feed conversion (Dominguez-vara *et al.,* 2009; Olivia-Teles and Goncalves 2001; Holtshausen and Beauchemin 2010; Moallem *et al.,* 2009; Mohamed *et al.,* 2009; Tripathi and Karim 2010). Several other yeasts like *Rhodosporidium paludigenum* (Yang *et al.,* 2010), *Candida* sp (Mahnken *et al.,* 1980; Sajeevan *et al.,* 2006; Sarlin and Philip 2011), *Phaffia rhodozyma* (Akiba *et al.,* 2001; Bjerkeng *et al.,* 2007; Johnson *et al.,* 1980; Sanderson and Jolly 1994), *Debaryomyces hansenii* (Sarlin and Philip 2011) and *Yarrowia lipolytica* (Hatlen *et al.,* 2012) are also beneficial to animal growth and performance when supplemented into animal feeds. Organic forms of minerals like selenium, chromium, iron or zinc for animal nutrition

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can be provided by *S. cerevisiae* (Dominguez-vara *et al.,* 2009; Fokkink *et al.,* 2009; Schrauzer 2006; Wang *et al.,* 2009), *Candida* sp., *Kluyveromyces* or *Pichia* sp. (Paš *et al.,* 2007; Roepcke *et al.,* 2011). Finally, yeast cell wall polysaccharides like β-D glucans and α-D-mannans (Mannan Oligosaccharide MOS) are also beneficial to animal health.

Carotenoids producing *P. rhodozyma* yeast cells are additives in poultry and aquaculture feeds (Jacobson *et al.,* 2003; Johnson *et al.,* 2010) and are beneficial to animal health (Amar *et al.,* 2004; Bjerkeng *et al.,* 2007; Sanderson and Jolly 1994; Takimoto *et al.,* 2007). According to the U.S. Food and Drug Administration, the dried and killed *P. rhodozyma* cells are permitted as a salmonid feed colorant to provide up to 80mg/kg of astaxanthin in the finished feed (USFDA; 21 CFR Section 73.355; Barrows *et al.,* 2003). To provide natural carotenoids in animal feeds, Ananda and Vadlani (2010a) developed the carotenoid fermentation of corn whole stillage, a coproduct of corn ethanol predominantly used as animal feed. Monoculture and mixed culture fermentation of red yeasts *P. rhodozyma* and *Sporobolomyces roseus* of corn whole stillage not only provided carotenoids but also enriched crude fat and polyunsaturated fatty acid by up to 81% and contained 77%-less fiber (Ananda and Vadlani 2010b). Similar carotenoid enrichment of several animal feeds like soybean products, rice bran and wheat bran, resulted in highest astaxanthin and β-carotene concentrations of 80 μg/g and 836 μg/g in *P. Rhodozyma*-fermented full fat rice bran and *S. roseus*-fermented full fat soy flour respectively (Ananda and Vadlani 2011). The aim of this study was to qualitatively and quantitatively characterize nutrients in the highest β-carotene producing *S. roseus*-fermented full fat soy flour and highest astaxanthin producing *P. rhodozyma*-fermented full fat rice bran. The effect of red yeast fermentation on anti-nutritional factor namely trypsin inhibitor (TI) in soybean flour and levels of yeast cell wall polysaccharides in the resultant animal feed were also evaluated.

MATERIALS AND METHODS

The samples generated for Ananda and Vadlani (2011) were nutritionally profiled in this study. The details of sample preparation, media preparation and fermentation conditions are outlined in Ananda and Vadlani (2010a, 2011).

Nutrition Analysis: Nutrition composition analyses of the fermented samples and controls were conducted to include total amino acid profile, total fatty acid profile, crude fat and protein, NDF and ADF and P, S and K. Further, samples were also profiled for glucosamine, glucose and mannose and the latter two were used to calculate yeast cell wall polysaccharides—glucans, mannans respectively according to Friemund *et al.* (2005) and soy flour samples were sampled for anti-nutritional factor, trypsin inhibitor. Samples from two replicates were pooled before analysis. Statistical analyses is not provided since the main objective of this study was to quantitatively and qualitatively estimate the nutrients in the highest astaxanthin yielding *P. rhodozyma*-fermented full fat rice bran and highest β-carotene yielding *S. roseus*-fermented full fat soy flour. Nutrient estimates from other treatments are provided only as a reference. About 5 g of each representative sample from each treatment was analyzed at Agricultural Experiment Station Chemical Laboratories, University of Missouri (Columbia, MO) for %NDF (JAOAC 56, 1352-1356, 1973), %ADF (AOAC Official Method 973.18 (A-D), 2006), total amino acid profile (AOAC Official Method 982.30E (a, b, c), chapter 45.3.05 (17)), total fatty acid profile (AOAC Official Method 996.06, AOCS Official Method Ce2-66, AOAC Official Method 965.49, AOAC Official Method 969.33 (17)), crude fat (acid hydrolysis, AOAC Official Method 954.02 (17)) and crude protein (Kjeldahl method, AOAC Official Method 984.13 (A-D) (17)). Estimation of % P, K and S was conducted at the Analytical Laboratory, Department of Animal Science and Industry, Kansas State University (Manhattan, KS).

RESULTS

The nutrition profiles of maximum carotenoid yielding *S. roseus*-fermented soy flour and *P. rhodozyma*-fermented rice bran were of interest. Overall, yeast fermentations resulted in reduction of protein and fiber in soy flour and rice bran, and enhancement of fat in rice bran (Tables 1 and 2) and reduction in crude fat in fermented soy flour samples (Table 1). In fact, *S. roseus*-fermented soy flour exhibited the least reduction in crude protein, fat, and its fiber reduction was three times lesser than that of *P. rhodozyma*-fermented soy flour (Table 1). *P. rhodozyma*-fermented rice bran exhibited the highest reduction in crude protein and crude fiber, and the least increase in crude fat (Table 2). Trypsin-inhibitor in the control (unfermented and unautoclaved *ie.,* raw) soy flour was 49,500 TIU/g and was reduced by 85% to 7,550 TIU/g by autoclaving alone (without any fermentation), and to indeterminate levels in both *S. roseus* and *P. rhodozyma* treatments (Table 1). Yeast polysaccharides like mannans and glucans absent in control soy flour were detected in the fermented samples with around 2.5% mannan and glucan each in *S. roseus* fermentation.

Table 1. Nutrition profile of *S. roseus*-fermented full fat soy flour (FFSF) which produced maximum β-carotene*.

Components [a]	Control	Mixed culture	*P. rhodozyma*	*S. roseus*
%Crude Protein [b]	35.81	30.98 (↓13.5%)	22.87 (↓36%)	30.95 (↓13.5%)
%Crude Fat [c]	16.26	14.2 (↓12%)	9.67 (↓41%)	14.1 (↓12%)
%Crude fiber	5.23	4.87 (↓12%)	3.34 (↓36%)	4.59 (↓7%)
%NDF	9.72	10.49 (↑8%)	7.3 (↓25%)	10.34 (↑6%)
%ADF	5.79	7.44 (↑28.5%)	2.64 (↓54%)	5.18 (↓10.5%)
%N	5.7	5.0 (↓12%)	3.7 (↓35%)	5.0 (↓12%)
%P	0.45	0.56 (↑24%)	0.4 (↓11%)	0.56 (↑24%)
%K	1.64	1.55 (↓5.5%)	1.19 (↓27%)	1.54 (↓6%)
%Mannan	-	3.23	1.01	2.38
% Glucann	-	3.52	2.43	2.48
%Glucosamine	-	0.3	1.96	4.61
Trypsin Inhibitor (TIU/g) [g]	49,500 [d] 7,550 [e]	1,456 (↓81%) [f]	n.d	n.d

*836.55μg/g as detailed in Ananda and Vadlani (2011).

[a] Numbers in parentheses indicate the % increase (↑) or decrease (↓) compared to the control; Maximum increase or decrease is boldfaced; n.d not-detected; [b] Kjeldahl; [c] Acid hydrolysis; [d] Trypsin inhibitor in unautoclaved control sample of full fat soy flour; [e] Trypsin inhibitor in autoclaved control sample of full fat soy flour reduced by 85% compared to unautoclaved control; [f] % reduction compared to autoclaved control sample [e]; [g] TIU/g converted to mg/g according to Stauffer (1990).

Table 2. Nutrition profile of *P. rhodozyma*-fermented full fat rice bran (FFRB) which produced maximum astaxanthin*.

Components [a]	Control	Mixed culture	*P. rhodozyma*	*S. roseus*
%Crude Protein [b]	13.46	12.32 (↓8%)	12.1 (↓10%)	12.78 (↓5%)
%Crude Fat [c]	15.91	25.94 (↑63%)	20.64 (↑30%)	24.78 (↑56%)
%Crude fiber	7.71	6.31 (↓18%)	5.81 (↓25%)	5.97 (↓23%)
%NDF	18.35	13.48 (↓26.5%)	13.53 (↓26%)	12.85 (↓30%)
%ADF	9.59	6.49 (↓32%)	6.31 (↓34%)	5.8 (↓40%)
%N	2.2	2.0 (↓9%)	1.9 (↓14%)	2.0 (↓9%)
%P	1.95	1.68 (↓14%)	1.74 (↓11%)	1.71 (↓12%)
%K	1.34	1.53 (↑14%)	1.26 (↓6%)	1.27 (↓5%)
%Mannan	-	2.75	1.1	2.5
% Glucann	-	5.65	4.77	4.93
%Glucosamine	-	0.13	0.37	0.42

*80.42 μg/g in Ananda and Vadlani (2011).

[a] Numbers in parentheses indicate the % increase (↑) or decrease (↓) compared to the control; Maximum increase or decrease is boldfaced; [b] Kjeldahl; [c] Acid hydrolysis.

The amino sugar, glucosamine found lacking in the control was found to be the highest in *S. roseus* treatment at 4.6% (Table 1). Among all three treatments, least amount of mannan (1.1%), and glucan (4.7%) were found in *P. rhodozyma*-fermented rice bran with 0.37% glucosamine (Table 2).

The least amino acid reduction of 19% was seen in *S. roseus*-fermented soy flour and (Table 3) whereas a maximum reduction of 17% in amino acid content was seen in *P. rhodozyma* fermented rice bran (Table 4). In both substrates, the highest reduction in total amino acids was seen in *P. rhodozyma* fermentation.

Table 3. Amino acid profile of profile of *S. roseus*-fermented full fat soy flour (FFSF) which produced maximum B-carotene*.

Amino acids [a]	w/w%			
	Control	Mixed culture	*P. rhodozyma*	*S. roseus*
Taurine	0.05	0.06	0.05	0.06
Hydroxyproline	0.06	0.25	0.44	0.23
Aspartic Acid	3.73	2.87	1.9	2.92
Threonine	1.28	1.18	1.12	1.17
Serine	1.33	1.21	1.14	1.18
Glutamic Acid	5.86	4.91	1.39	4.68
Proline	1.64	1.29	0.77	1.34
Lanthionine	0.17	0.15	0.78	0.16
Glycine	1.5	1.52	0.99	1.58
Alanine	1.53	1.45	0.91	1.46
Cysteine	0.53	0.6	0.29	0.61
Valine	1.83	1.41	1.52	1.41
Methionine	0.47	0.37	0.26	0.37
Isoleucine	1.66	1.08	1.29	1.09
Leucine	2.68	1.82	1.7	1.82
Tyrosine	1.23	0.86	0.7	0.88
Phenylalanine	1.74	1.06	1.08	1.07
Hydroxylysine	0.01	0.13	0	0.22
Ornithine	0.03	0.23	0.06	0.24
Lysine	2.29	1.63	1.4	1.64
Histidine	0.98	0.76	0.72	0.79
Arginine	2.48	1.91	1.5	2.01
Tryptophan	0.43	0.33	0.27	0.35
Total	33.51	27.08 (↓19%)	20.28 (↓39%)	27.28 (↓19%)

*836.55μg/g as detailed in Ananda and Vadlani (2011). [a]Numbers in parentheses indicate the % decrease (↓) compared to the control and the maximum decrease is boldfaced.

Table 4. Amino acid profile of *P. rhodozyma*-fermented full fat rice bran (FFRB) which produced maximum astaxanthin*.

Amino acids [a]	w/w%			
	Control	Mixed culture	*P. rhodozyma*	*S. roseus*
Taurine	0.01	0.03	0.02	0.02
Hydroxyproline	0.05	0.18	0.17	0.2
Aspartic Acid	1.09	1.03	0.92	1.07
Threonine	0.46	0.55	0.52	0.54
Serine	0.46	0.55	0.49	0.56
Glutamic Acid	1.63	1.25	1.02	1.38
Proline	0.52	0.52	0.47	0.57
Lanthionine	0.06	0.06	0.08	0.07
Glycine	0.72	0.74	0.65	0.83
Alanine	0.81	0.74	0.67	0.75
Cysteine	0.27	0.25	0.2	0.29
Valine	0.75	0.68	0.66	0.67
Methionine	0.24	0.19	0.19	0.19

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Isoleucine	0.48	0.44	0.44	0.43
Leucine	0.9	0.84	0.79	0.83
Tyrosine	0.38	0.34	0.3	0.35
Phenylalanine	0.54	0.46	0.42	0.46
Hydroxylysine	0.03	0.08	0.03	0.06
Ornithine	0.01	0.02	0.01	0.02
Lysine	0.67	0.66	0.62	0.65
Histidine	0.41	0.3	0.31	0.3
Arginine	1.11	0.66	0.63	0.67
Tryptophan	0.1	0.1	0.07	0.11
Total	11.7	10.67 (↓9%)	9.68 (↓17%)	11.02 (↓6%)

*80.42 µg/g in Ananda and Vadlani (2011). [a]Numbers in parentheses indicate the % decrease (↓) compared to the control and the maximum decrease is boldfaced.

Tables 5 and 6 outline the fatty acid profiles in carotenoid-enriched full fat soy flour and rice bran respectively. Even though percent crude fat was reduced in the fermented soy flour samples compared to the control there was enhancement of several fatty acids (Table 5). The most abundant fatty acids accounting to more than 2% of total fat in full fat soy flour were linoleic acid, oleic acid, palmitic acid, linolenic and stearic acid in that order and the order remained unchanged even in the fermented treatments albeit changes in concentrations of fatty acids compared to the control (Table 5).

Table 5. Fatty acid profile of profile of *S. roseus*-fermented full fat soy flour (FFSF) which produced maximum B-carotene*.

Fatty Acid a (% of total fat)	Control	Mixed culture	P. rhodozyma	S. roseus
Myristic (14:0)	0.16	0.63	0.13	0.74
Myristoleic (14:1)	0	0	0	0
(C15:0)	0.03	0.16	0.07	0.14
Palmitic (16:0)	10.78	14.0	12.33	13.94
Palmitoleic (16:1)	0.1	0.9	0.11	0.98
(17:0)	0.12	0.11	0.17	0.1
(17:1)	0.07	0.11	0.08	0.11
Stearic (18:0)	4.68	2.48	6.29	2.15
Elaidic (18:1t9)	0.04	0.12	0.06	0.12
Oleic (18:1n9)	17.99	33.17	22.28	33.09
Vaccenic (18:1n7)	1.3	0	0.93	0
Linoleic (18:2)	53.3	38.41	48.18	38.51
Linolenic (ω18:3)	9.46	5.78	6.98	6.12
(ω18:4)	0.03	0	0.03	0
Arachidic (20:0)	0.42	0.34	0.51	0.29
(20:1n9)	0.15	0.4	0.12	0.36
(20:3 ω3)	0	0	0	0
Arachidonic (20:4n6)	0	0	0	0
Arachidonic (20:4 ω3)	0	0	0	0
(20:5 ω3; EPA)	0	0	0	0
Docosanoic (22:0)	0	0	0.01	0
Erucic (22:1n9)	0	0.07	0.01	0.07
(22:5 ω3; DPA)	0	0	0	0
(22:6 ω3; DHA)	0	0.08	0.02	0.08
Lignoceric (24:0)	0.16	0.56	0.27	0.62
Nervonic (24:1n9)	0	0.04	0	0.04
Crude Fat (by acid hydrolysis)	16.26	14.2 (↓12%)	9.67 (↓41%)	14.1 (↓12%)

*836.55µg/g as detailed in Ananda and Vadlani (2011). [a] in parentheses indicate the % decrease (↓) compared to the control and the maximum reduction is boldfaced.

Among the abundant fatty acids, palmitic acid and oleic acid levels were enhanced in all yeast treatments with maximum enhancement of 31% palmitic acid and 84% oleic acid in *S. roseus*-fermented soy flour (and mixed fermentation) compared to control. Interestingly, *S. roseus*-fermented soy flour (and mixed culture fermentation) showed spectacular enhancement of fatty acids accounting less than 1% of total fat: 880% yield enhancement for palmitoleic acid, 363% of myristic acid, 200% eladic acid and 288% lignoceric acid (Table 5). In the case of rice bran, three fatty acids namely oleic, linoleic and palmitic in that order accounted for 92% of total fat in full fat rice bran (Table 6). The abundant fatty acids in control rice bran were oleic acid>linoleic acid>palmitic acid>stearic acid>linolenic acid which was similar to that in mixed culture and *S. roseus* fermentation. However, in *P. rhodozyma* fermentation the order remained the same except that linoleic acid was greater than oleic acid (Table 6). Although *P. rhodozyma*-fermented rice bran exhibited 63% enhancement of crude fat, only a few of the major fatty acids like stearic acid and linoleic acid were enhanced by 389% and 9% respectively.

Table 6. Fatty acid profile of *P. rhodozyma*-fermented full fat rice bran (FFRB) which produced maximum astaxanthin*.

Fatty Acid [a] (% of total fat)	Control	Mixed culture	*P. rhodozyma*	*S. roseus*
Myristic (14:0)	0.49	0.6	0.18	0.58
Myristoleic (14:1)	0	0	0	0
(C15:0)	0.04	0.07	0.07	0.07
Palmitic (16:0)	16.35	15.62	16.42	14.96
Palmitoleic (16:1)	0.21	0.78	0.18	0.8
(17:0)	0.06	0.09	0.22	0.08
(17:1)	0.05	0.11	0.07	0.13
Stearic (18:0)	1.75	2.93	8.56	2.12
Elaidic (18:1t9)	0.05	0.13	0.08	0.13
Oleic (18:1n9)	40.68	44.58	31.03	46.82
Vaccenic (18:1n7)	0	0	0	0
Linoleic (18:2)	34.86	28.76	38.09	27.68
Linolenic (ω18:3)	1.42	2.29	1.15	2.46
(ω18:4)	0.04	0	0	0
Arachidic (20:0)	0.76	0.57	0.87	0.54
(20:1n9)	0.54	0.51	0.33	0.56
(20:3 ω3)	0	0	0	0
Arachidonic (20:4n6)	0	0	0	0
Arachidonic (20:4 ω3)	0	0	0	0
(20:5 ω3; EPA)	0	0	0	0
Docosanoic (22:0)	0	0	0	0
Erucic (22:1n9)	0.04	0	0.03	0
(22:5 ω3; DPA)	0	0	0	0
(22:6 ω3; DHA)	0.16	0.11	0.1	0.11
Lignoceric (24:0)	0.85	0.87	0.76	0.89
Nervonic (24:1n9)	0.03	0.03	0	0.05
Crude Fat (by acid hydrolysis)	15.91	25.94 (↑**63** %)	20.64 (↑30%)	24.78 (↑56%)

*80.42 μg/g in Ananda and Vadlani (2011). [a]Numbers in parentheses indicate the % increase (↑) compared to the control and the maximum increase is boldfaced.

DISCUSSION

Direct incorporation of carotenoid feed supplements like astaxanthin and β-carotene by red yeast fermentation of corn whole stillage was demonstrated by Ananda and Vadlani (2010a). Similar fermentation of nine agricultural products used as animal feed found that *S. roseus*-fermented full fat soy flour and *P. rhodozyma*-fermented full fat rice bran respectively yielded the highest β-carotene and astaxanthin (Ananda and Vadlani 2011). Overall, carotenoid-fermented corn whole stillage (98 to 279 µg/g; Ananda and Vadlani (2010a)), *S. roseus*-fermented full fat soy flour (837µg/g) or *P. rhodozyma*-fermented full fat rice bran (80µg/g) contained carotenoids well over the prescribed dietary dosage of 1 to 120µg/g feed (An *et al.,* 2006; Hayek 2000) which can be used to make feed blends to provide the appropriate quantity of carotenoids. Carotenoid production in fermented-corn whole stillage, *S. roseus*-fermented full fat soy flour or *P. rhodozyma*-fermented full fat rice bran was accompanied by reduction in crude fiber and crude protein with varying levels of reduction. Contrastingly, yeast fermented rice bran enhanced crude fat similar to that observed in corn whole stillage (Ananda and Vadlani, 2010b), but was decreased in full fat soy flour. So, to provide adequate amount of carotenoids and other nutrients, *S. roseus*-fermented full fat soy flour or *P. rhodozyma*-fermented full fat rice bran can be used in 'feed blends' based on the dietary requirements of the animals (Ananda and Vadlani 2010a, 2011).

Irrespective of the effect of yeast fermentation on crude fat, the relative abundance of fatty acid composition of *P. rhodozyma* remained similar: the most abundant fatty acids were linoleic acid> oleic acid> palmitic acid>stearic acid>linolenic acid in soy flour, rice bran and whole stillage (Ananda and Vadlani 2010b). This was similar to that observed in commercial *P. rhodozyma* cells described in Sanderson and Jolly (1994). The abundance of fatty acids in *S. roseus*, unlike *P. rhodozyma* was variable: for example, in soy flour fermentation the order of fatty acid abundance was linoleic acid>oleic acid>palmitic acid>stearic acid whereas in rice bran the order was oleic acid>linoleic acid>palmitic acid> linolenic acid>stearic acid and in corn whole stillage (Ananda and Vadlani 2010b) it was vaccenic acid>linoleic acid>palmitic acid. Libkind *et al.* (2008) found that linoleic acid>oleic acid>palmitic acid were the major fatty acids in *Sporobolomyces patagonicus* and concluded that growth media influenced the fatty acid composition and abundance of red yeasts which is confirmed from the present study and that of Ananda and Vadlani (2010b). Nutrition profiling of mixed culture fermentation closely resembled that of *S. roseus* in all three substrates including whole stillage carotenoid fermentation outlined in Ananda and Vadlani (2010b). Fermentation media composition seems to influence *S. roseus* fatty acid composition more than that in *P. rhodozyma*.

Effect of red yeast fermentation on anti-nutrition factor like trypsin inhibitor: According to the soybean meal specifications mandated by the U.S. National Oilseed Processors Association (NOPA), the permissible limit of trypsin inhibitor (TI) is less than 4mg/g of soybean meal. Typically, raw soybean contains 20.9 to 31.1mg/g of TI, and in low-TI soybean varieties it could be as low as 9.9 mg/g (Herkelman *et al.,* 1992; Vandergrift *et al.,* 1983). Typically, TI interferes with the action of enzymes trypsin and chymotrypsin and impairs protein digestion especially in swine, poultry and fish (Liener 1994; Olli *et al.,* 1994; Palliyeguru *et al.,* 2011; van den Ingh *et al.,* 1991). So, heating soybean especially with moist heat like steaming or autoclaving (Combs *et al.,* 1967, Herkelman *et al.,* 1992; Khattab and Arntfield 2009; Mateos *et al.,* 2002; Reddy and Pierson 199; Vandergrift *et al.,* 1983), microbial fermentations (Barapama and Simard 1994, Hoffman et al., 2003, Khattab and Arntfield 2009; Meijer *et al.,* 1995; Osman 2004) and combination of heating and microbial fermentation (Reddy and Pierson1994) can reduce TI levels: the red yeast fermentation of soybean flour for animal feed not only enriched the feed with carotenoids required for animal health, but also eliminated the antinutritional factor trypsin-inhibitor that can interfere with animal nutrition.

Yeast cell wall components: In this study, carotenoid-enriched soy flour and rice bran animal feed contained 1.01 to 3.23% and 1.1 to 2.75% mannan respectively, and 2.43 to 3.52% and 4.77 to 5.65% glucans respectively. The routinely used dosage for both polysaccharides is 0.1 to 0.25% *ie.,* 1.0 to 2.5kg/ton animal feed in poultry, swine, cattle feed or aquaculture feed based on the animal growth phase (Center for Food and Nutrition Policy TAP Review 2002; Cook *et al.,* 2002). Yeast cell wall polysaccharides like β-D glucans and α-D-mannans promote animal health by immunomodulation, blocking bacterial adhesion in the gut thereby preventing bacterial infections and by adsorbing mycotoxins in animal feed and also by inhibiting their toxic effects, enhance weight gain, improve quality of milk in cattle and enhance feed conversion efficiency (Hayen and Pollmann 2001; Kogan and Kochar 2007; Noeck *et al.,* 2011, Zeković *et al.,* 2005). Since red yeast fermentations of full fat soy flour and rice bran yield 10- to 20-times the recommended dosage of glucans and mannans, the carotenoid-enriched feed can be used to make feed blends to provide adequate concentration of yeast cell wall nutrients in animal nutrition.

Glucosamine is a structural component of cartilage. Glucosamine is often used as a nutraceutical for horses, pet animals and humans to relieve osteoarthritic conditions although the health benefits largely remain inconclusive (McFarlan *et al.,* 2004; Igarashi *et al.,* 2011; Pearson and Lindinger 2009). Since fungal cell wall chitin is made up of glucosamine, fungal or yeast fermentations of wild or genetically modified strains can yield glucosamine and have the potential to overcome the disadvantages associated with present production methods from crustacean shells (Deng *et al.,* 2012; Hsieh *et al.,* 2007; McFarlan *et al.,* 2004; Zhang *et al.,* 2012). In the present study, the red yeast fermented, carotenoid-enriched soy flour and rice bran contained 0.3 to 4.6% and 0.13 to 0.42% glucosamine respectively, and in both substrates *S. roseus* yielded the highest glucosamine levels.

CONCLUSIONS

High levels of carotenoids in *S. roseus*-fermented soy flour and *P. rhodozyma*-fermented rice bran are accompanied by reduced fiber, protein and amino acids, and respective enhancement or reduction of crude fat; almost complete elimination of antinutritional factor trypsin inhibitor in soy flour; and enrichment of health promoting yeast cell wall polysaccharides. Levels of carotenoids and yeast cell wall polysaccharides produced in excess of the daily dietary needs of animals by red yeast fermentation easily allows production of feed blends to provide adequate nutrients based on animal dietary requirements. *P. rhodozyma* and *S. roseus* fermentations of commonly used animal feeds are valuable in providing a suite of nutrients that are proven to improve animal health.

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