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An Investigation on Optimization and Fermentative Changes During the Production of Food Bio-Colours through Solid State Fermentation of Wheat bran by *Monascus Purpureus* (Mtcc 410) Strain

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Natural pigments are an important alternative to potentially harmful synthetic dyes used as colourant in foods. The toxicity problems caused by those of synthetic colours to the consumers have created a mounting interest towards natural colours. The food bio-colours are gaining importance and have become the focus of attention of many scientists all over the world. The feasibility of wheat bran as a substrate for production of food bio-colour by *Monascus purpureus* **(MTCC 410) strain in SSF was investigated by optimizing the fermentation conditions. The higher yield of red, orange and yellow bio-colours achieved were 126.76, 76.76 and 56.05 OD Units/g dms respectively with wheat bran at optimized process parameters viz. 75% (v/w) initial moisture content, 0.3-0.4 mm particle size, pH 6, incubation at 30°C, inoculation with 3% (spore suspension) of 5 days old culture and an incubation period of 9 days with supplementation as glucose (3% w/w) and peptone (1% w/w) as a carbon and nitrogen source, respectively. The enhanced yield of bio-colours indicated that wheat bran has good potentiality for the production of food bio-colours through SSF.**

Key words: Bio-colours, *Monascus,* Solid State Fermentation, Wheat bran.

With the advent of strict legislative regulations and growing awareness among the consumers about food safety, bio-colours have become the choice in processed foods being safer than their synthetic counterparts. It is great advantageous to use microbes for food bio-colour production due to their intrinsic properties of high growth rate, no seasonal variation, high production rate and ease of manipulation. The bio-colours have been produced from large number of bacterial, yeast and mold species which have some necessary features like capability to use a wide range of carbon and nitrogen sources, should have tolerance to pH, temperature, mineral concentration and possess moderate growth conditions, reasonable colour yield, should be non toxic and non pathogenic, must be easily separable from cell mass (Joshi *et al.,* 2003).

Monascus is probably a xerophillic fungus, which grows in a wide variety of natural substrates (Babhita *et al.,* 2004). Bio-colours from this fungus are widely used in food and pharmaceutical industries for therapeutic uses also (Kumar *et al.,* 2012). The solid state fermentation (SSF) approach gives high bio-colour productivity at a low cost when compared with liquid fermentation (Cavalcante *et al.,* 2008). The SSF process offers major advantages in the form of use of cost effective agro-industrial residues as substrate, uses simple instrumentation and ease in downstream processing of fermented media (Shaligram *et al*., 2009)

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Several materials such as jackfruit seed powder (Babhita *et al.,* 2006; Babhita *et al*., 2007); apple pomace (Attri and Joshi, 2005a,b; Joshi and Attri, 2006); cassava starch (Yongsmith *et al*., 1993); prickly pear juice (Hamdi *et al.,* 1996) and dairy milk (Kujumdzieva *et al.,*1997) have been exploited as substrates in SSF.

In recent years, the use of wheat and other cereal bran has gained importance in the formulation of various food products. Wheat bran is easily available and cheap agro-industrial residue which is good source of proteins, lipids, vitamins and minerals. The utilization of wheat bran helps in reducing the environmental pollution due to its disposal (Cristina and Eliana, 2009). Therefore, the present investigation was aimed to address the fermentative changes in wheat bran as a substrate during the production of food bio-colours through SSF process by using *Monascus purpureus* (MTCC 410). The present investigation assessed the influence of SSF condition for *Monascus purpureus* (MTCC 410) strain on the functional and physico-chemical properties of wheat bran intending to provide information about their use in food product formulation.

Materials and Methods

Microbial Strain

The freeze dried culture of *Monascus purpureus* (MTCC 410) strain was obtained from Institute of Microbial Technology, Chandigarh, India. The stock

culture was grown on potato dextrose agar (PDA) slants for 7 days at 30°C and maintained at 4°C and periodically sub-cultured after every 2 months.

Wheat bran

The fresh wheat bran was extracted from wheat NIAW 301 (Trimbak) variety by flour mill (Brabender make)

Preparation of inoculum

The *Monascus purpureus* (MTCC 410) strain was grown on PDA slants for 7 days at 30°C before use as inoculum.

Solid state fermentation

10 g of wheat bran was washed with tap water and subjected to SSF in Earlenmayer flask as per the procedure depicted in Fig.1. (Vanajakshi, 2006). The fermented wheat bran (10 g) was dissolved in 25 ml of different solvents (water, alcohol and hexane for orange, red and yellow bio-colour respectively) in flasks and these were kept in orbital shaker for 15 min at 100 rpm. The solvent was then filtered through Whatman filter paper. The filtrates from different samples were centrifuged at 2400 rpm for 10 min. and supernatants were collected and ODs recorded using spectrophotometer at λ_{500}, λ_{475} and λ_{375} for red, orange and yellow bio-colours respectively. Finally, the total bio-colour yield from the fermented substrate was expressed as the sum total of red, orange and yellow bio-colours in OD Units/g dry mouldy substrate (Johns and Stuart, 1991).

The wet fermented mouldy wheat bran (2g) was dried in hot air oven at 105°C for 4 h. The difference in the weight was recorded as the moisture content of mouldy wheat bran and weight of residue was recorded as weight of mouldy substrate (Vanajakshi, 2006).

Statistical analysis

The data obtained in the present investigation was statistically analysed for analysis of variance and completely randomized design (CRD) as per the method of Panse and Sukhatme (1989).

Results and Discussion

Proximate composition of wheat bran

The chemical analysis revealed that the wheat bran was composed of 57.98% carbohydrate, 14.24% protein, 4.21% fat, 8.97% fiber, 4.92% ash and 9.68% moisture with pH of 6.2 (Table 1). Cristina and Eliana (2009) reported that wheat bran found crude protein, crude fat, crude fiber, ash and moisture contents ranged between 13.8%, 5.2%, 5.2%, 6.3% and 9.4% respectively. More or less similar values for chemical composition of wheat bran were reported by Butt *et al.* (2010); Gamal *et al.* (2012); Hemanti matin *et al.* (2013) and Hadden *et al.* (2014).

Wheat bran can be considered as a suitable substrate for SSF due to presence of sufficient nutrients like carbohydrates, protein, fiber and minerals and due to this has attracted the scientist's world over and has become a major battle front to produce high value products through SSF.

Table 1. Proximate composition of fermented wheat bran

Chemical parameter	Measurement/Value	
	Unfermented	Fermented
Carbohydrates (%)	57.98	11.37
Protein (%)	14.24	19.37
Fat (%)	4.21	4.78
Crude fiber (%)	8.97	14.88
Ash (%)	4.92	5.91
Moisture (%)	9.68	43.69
pH	6.2	5.8

Each value represents average of three determinations

Yield profile of food bio-colours after optimized conditions

The optimization of medium components and fermentation parameters is of primary importance in any fermentation process. Wheat bran as a substrate in solid state fermentation was employed for optimization of fermentation parameters namely moisture content, particle size, incubation temperature, inoculum age, inoculum size, incubation time and initial pH of the medium as well as the extra supplementation of carbon and nitrogen sources were optimized to maximize the bio-colours yield. The result of optimization study proved that wheat bran has potential to be a substrate for the production of food bio-colours through SSF. Food bio-colours production by *Monascus purpureus* (MTCC 410) strain under SSF was influenced by physiological and chemical nature of the wheat bran and associated with growth of the *Monascus purpureus* (MTCC 410) strain.

Table 2. Effect of initial moisture content on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dms)			
	Red	Orange	Yellow	Total
TM_1	7.61	5.43	2.17	15.02
TM_2	12.61	7.88	4.73	25.21
TM_3	18.33	11.28	6.58	36.18
TM_4	22.26	14.55	8.56	45.38
TM_5	18.44	12.59	6.29	37.32
SE ±	0.15	0.15	0.16	0.18
CD at 5%	0.46	0.46	0.47	0.53

(TM_1=60%, TM_2=65%, TM_3=70%, TM_4=75% and TM_5=80%)

The higher yield of red, orange and yellow bio-colours obtained were 22.26 OD Units/g dms at 500 nm, 14.55 OD Units/g dms at 475 nm and 8.56 OD Units/g dms at 375 nm respectively at 75% (v/w) initial moisture content of wheat bran as compare to other levels of initial moisture (Table 2). The present findings are comparable with the findings reported by Gautam *et al*. (2002) and Perez-Guerra *et al*. (2003) for initial

moisture content of various agricultural wastes or by-products as a substrate in SSF. The moisture content of substrate plays a key role in fungal growth, enzyme activity and metabolite production in SSF (Yongsmith *et al.,* 2000; Wang *et al.,* 1975; Pandey 2003). The optimum initial moisture content favoured the mass transfer, intake of oxygen and release of carbon dioxide (Lotong and Suwanarit, 1990; Babitha *et al.,* 2007) and also facilitates effective absorption of the nutrients from the substrates for growth and metabolic activities (How and Ibrahim, 2004).

Table 3. Effect of particle size on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dms)			
	Red	Orange	Yellow	Total
TP_1	8.77	6.58	3.84	19.19
TP_2	14.55	8.08	5.39	28.02
TP_3	20.83	14.96	9.62	45.41
TP_4	38.34	22.58	14.71	75.63
TP_5	30.19	21.19	12.71	64.09
SE ±	0.17	0.16	0.37	0.21
CD at 5%	0.50	0.48	1.10	0.63

(TP_1=0.09-0.1 mm, TP_2=0.1-0.2 mm, TP_3=0.2-0.3 mm, TP_4=0.3-0.4 mm and TP_5=0.4-0.6 mm)

The wheat bran of particle size in between 0.3-0.4 mm was optimal for bio-colour yield. The peak yield of red, orange and yellow bio-colours were recorded as 38.34 OD Units/g dms at 500 nm, 22.58 OD Units/g dms at 475 nm and 14.71 OD Units/g dms at 375 nm respectively at 0.3-0.4 mm particle size. Generally, smaller substrate particles provide a larger surface area for microbial activity and thus it should be considered as a desirable factor for higher bio-colour production.

Table 4. Effect of temperature on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dms)			
	Red	Orange	Yellow	Total
T_1	17.93	15.76	4.35	38.04
T_2	22.44	19.23	7.48	49.07
T_3	30.36	22.45	11.45	64.26
T_4	16.42	26.68	14.94	58.04
T_5	12.39	15.63	19.60	47.62
SE ±	0.33	0.17	0.19	0.20
CD at 5%	0.99	0.52	0.57	0.60

(T_1=20°C, T_2=25°C, T_3=30°C, T_4=35°C and T_5=40°C)

However, too small particles may result in substrate agglomeration, which may interfere with aeration (due to less interparticle space) and thus may result in poor microbial growth and bio-colour yield. At the same time, larger particles provide better aeration efficiency (due to increased interparticle space), but provide limited surface for microbial activity (Domsch *et al.,* 1980). Among the several factors in solid state fermentation which are important for microbial growth activity, the substrate particle size is one of the most critical parameters (Pandey, 1991; Zadrazil and Puniya, 1995).

The yield obtained for red, orange and yellow bio-colours were 30.36 OD Units/g dms at 500 nm, 26.68 OD Units/g dms at 475 nm and 19.60 OD Units/g dms at 375 nm respectively at 30°C, 35°C and 40°C (Table 4). The total yield of bio-colours decreased drastically at higher temperatures due to the mesophillic nature of *Monascus* spp. The maintenance of an optimal process temperature is one of the major factors for getting higher yields of microbial metabolites. The temperature affects microbial cellular growth, spore formation, germination and microbial physiology, thus affecting bio-colour formation. Results are in agreement with Domsch *et al.* (1980) and Babitha *et al.* (2006) reported an optimum temperature of 30°C to 37°C for growth of *Monascus* spp.

Table 5. Effect of inoculum age on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dms)			
	Red	Orange	Yellow	Total
TG_1	19.89	12.70	7.95	40.34
TG_2	21.21	16.18	9.49	46.88
TG_3	30.98	18.48	10.33	59.78
TG_4	28.85	16.03	8.01	52.88
TG_5	26.94	13.47	6.47	46.88
SE ±	0.17	0.20	0.14	0.20
CD at 5%	0.52	0.60	0.42	0.61

(TG1=3 Days, TG2=4 Days, TG3=5 Days, TG4=6 Days and TG5=7 Days)

The results indicated (Table 5) that the wheat bran inoculated with 5 days old culture gave better yield of bio-colour i.e. red colour (30.98 OD Units/g dms at 500 nm), followed by orange colour (18.48 OD Units/ g dms at 475 nm) and yellow colour (10.33 OD Units/ g dms at 375 nm) as shown in Table 5. An increased in inoculum age resulted in decreased mycelial growth. Amongst several fungal physiological properties, the inoculum age usually plays an important role in fungal activity (Glazebrook *et al.,* 1992; Bae *et al.,* 2000).

Table 6. Effect of inoculum size on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dms)			
	Red	Orange	Yellow	Total
TS_1	19.69	12.50	5.68	38.07
TS_2	21.21	16.18	9.49	46.88
TS_3	33.61	18.32	12.41	64.12
TS_4	29.38	19.23	12.82	61.43
TS_5	30.98	18.48	10.33	59.78
SE ±	0.18	0.15	0.14	0.22
CD at 5%	0.54	0.45	0.44	0.65

(TS_1=1%, TS_2=2%, TS_3=3%, TS_4=4% and TS_5=5%)

The results (Table 6) indicated that inoculating medium with 3% spores suspension reported considerably higher yield of red bio-colour (33.61 OD Units/g dms at 500 nm), followed by orange bio-colour (18.32 OD Uniits/ g dms at 475 nm) and yellow bio-colour (12.41 OD Units/ g dms at 375 nm). The lower

levels of inoculum resulted in insufficient biomass and lower yield of bio-colour, whereas too much inoculum produced excessive biomass and depleted the nutrients required for bio-colour formation (Babitha *et al.,* 2006). The results are in agreement with previous studies (Babitha *et al.*, 2006; Chakradhar *et al.,* 2009). A suitable inoculum size was needed for highest yield of bio-colours (Ramachandran *et al.*, 2004).

Table 7. Effect of incubation time on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dms)			
	Red	Orange	Yellow	Total
TT_1	15.77	9.01	4.70	29.28
TT_2	23.78	17.15	4.98	45.91
TT_3	29.89	16.30	6.52	52.64
TT_4	34.48	19.40	11.31	65.19
TT_5	32.59	17.63	9.62	60.03
SE ±	0.17	0.15	0.22	0.19
CD at 5%	0.50	0.44	0.66	0.58

(TT_1=3 Days, TT_2=5 Days, TT_3=7 Days, TT_4=9 Days and TT_5=12 Days)

The higher yield of red bio-colour on 9th day of fermentation was 34.48 OD Units/g dms at 500 nm, while yield of orange and yellow bio-colours were 19.40 OD Units/g dms at 475 nm and 11.31 OD Units/g dms at 375 nm respectively (Table 7). Velmurugan *et al*. (2011) reported the maximum yield of red bio-colour 33.42 OD Units/g dms, while yellow bio-colours it was 15.28 OD Units/g dms on 7th day of fermentation by *Monascus purpureus* (KACC 42430). The production of bio-colour decreased after 9th day of incubation which might be due to the decline in growth of the fungus due to the depletion of medium ingredients. More or less similar results were reported by Carvalho *et al*. (2005)

Table 8. Effect of pH on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dms)			
	Red	Orange	Yellow	Total
TH_1	7.74	9.96	13.27	30.97
TH_2	10.33	13.04	16.30	39.67
TH_3	14.96	20.83	13.89	49.68
TH_4	29.66	19.60	12.71	61.97
TH_5	21.01	15.63	9.70	46.34
SE ±	0.16	0.17	0.20	0.21
CD at 5%	0.48	0.50	0.59	0.62

(TH_1=3, TH_2=4, TH_3=5, TH_4=6 and TH_5=7)

The results showed that red bio-colour yield was maximum (29.66 OD Units/g dms at 500 nm) at pH 6 and orange bio-colour (20.83 OD Units/g dms at 475 nm) was maximum at pH 5 (Table 8). These results are consistent with Babitha *et al.* (2006), who reported maximum bio-colour production by *Monascus purpureus* (MTCC 410) at pH 4.5 to 7.5, with jack fruit seed powder as substrate in solid state fermentation. Yongsmith *et al.* (2000) reported that a lower pH promotes synthesis of yellow bio-colour, whereas a higher pH red bio-colour.

Addition of glucose, maltose, fructose, sucrose and lactose with concentration (3% w/w) to the wheat bran medium showed varying yield of bio-colour. Among all the sugars used, fructose resulted in highest total yield of bio-colour (182.48 OD Units/g dms), followed by glucose (173.75 OD Units/g dms), maltose (155.56 OD Units/g dms), sucrose (139.22 OD Units/g dms) and lactose (120.77 OD Units/g dms). The addition of fructose (3% w/w) in basal wheat bran substrate medium also improved the yield of red, orange, yellow and total bio-colour upto values of 85.94 OD Units/g dms at 500 nm, 61.94 OD Units/g dms at 475 nm, 34.60 OD Units/g dms at 375 nm and 182.48 OD Units/g dms respectively. The results obtained are almost identical to that reported by Omamor *et al*. (2008). They observed that *Monascus purpureus* utilized fructose very well compared to *Monascus ruber* in date palm fruits or residue.

Table 9. Effect of carbon sources on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dms)			
	Red	Orange	Yellow	Total
TC_1	81.88	54.38	37.50	173.75
TC_2	70.14	56.94	28.47	155.56
TC_3	85.94	61.94	34.60	182.48
TC_4	71.35	49.23	18.64	139.22
TC_5	64.69	38.93	17.15	120.77
SE ±	0.35	0.29	0.39	0.58
CD at 5%	1.04	0.87	1.16	1.73

(TC_1=Glucose, TC_2=Maltose, TC_3=Fructose, TC_4=Sucrose and TC_5=Lactose)

It was observed that peptone resulted in enhanced production of bio-colour followed by MSG (Table 10). The higher yield of red, orange, yellow and total bio-colours of 126.76 OD Units/g dms at 500 nm, 76.76 OD Units/g dms at 475 nm, 56.05 OD Units/g dms at 375 nm and 259.38 OD Units/g dms respectively were obtained using peptone (1% w/w) as a nitrogen source. Subhasree *et al*. (2011) also reported that peptone was found to yield maximum pigment and also observed that organic nitrogen sources gave a better yield than inorganic sources. Similar results were reported by Babhita *et al*. (2006) with Jackfruit seed powder supplemented with peptone as a organic nitrogen source for the production of water-soluble *Monascus* pigments.

Bio-colours yield after optimized conditions

The higher yields of red, orange, yellow and total bio-colours were 126.76 OD Units/g dms, 76.76 OD Units/g dms, 56.05 OD Units/g dms and 259.38 OD Units/g dms respectively achieved with wheat bran medium at optimized process parameters including 75% (v/w) initial moisture content, 0.3-0.4 mm particle size, pH 6, 30°C incubation temperature with 3% inoculum of 5 days old culture and an incubation period of 9 days under the influence of fructose (3% w/w) and peptone (1% w/w) as a carbon and

nitrogen source respectively. Solid state fermentation of wheat bran substrate medium gave a superior total yield of 259.38 OD Units/g dms which is about 3-fold higher than the highest yield reported in the previous literature. Needless to say, our process is a potent candidate for commercially viable production of food bio-colours. Food bio-colours production by *Monascus purpureus* (MTCC 410) strain under SSF was influenced by physiological and chemical nature of the wheat bran and associated with growth of the *Monascus purpureus* (MTCC 410) strain.

Table 10. Effect of nitrogen sources on bio-colours yield

Treatments	Bio-colour yield (OD Units/g dms)			
	Red	Orange	Yellow	Total
TN_1	126.76	76.76	56.05	259.38
TN_2	85.73	54.76	51.77	192.26
TN_3	93.19	63.06	52.46	208.71
TN_4	111.25	72.50	50.63	234.38
TN_5	118.06	68.75	60.42	247.22
SE ±	0.21	0.26	0.24	0.27
CD at 5%	0.62	0.77	0.71	0.81

(TN_1=Peptone, TN_2=Urea, TN_3=Ammonium sulphate, TN_4=Yeast extract and TN_5=MSG)

Proximate composition of fermented wheat bran

The data pertaining to various chemical properties of fermented wheat bran is depicted in Table 1. The proximate composition of fermented wheat bran plays an important role for deciding its nutritional, functional and storage qualities. It was observed (Table 1) that carbohydrates content of fermented wheat bran was 11.37%, protein 19.37%, fat 4.78%, fiber 14.88%, ash 5.91%, moisture 43.69% with pH of 5.8.

The results showed that the reduction of total carbohydrates from 57.98% in the wheat bran substrate to 11.37% on day 9. The carbohydrate content present in unfermented wheat bran reduced by about 80.38% in fermented one. The total carbohydrate content showed a steady and significant decrease during the fermentation possibly due to the breakdown of carbohydrate by the action of fungal amylases, releasing the simple and utilizable carbohydrate molecules for its metabolic activities. *Monascus purpureus* (MTCC 410) produced the highest protein content in the fermented wheat bran which was observed after 9 days of fermentation and the increase was around 36.02% in relation to unfermented bran. Similar results were reported by Cristina and Eliana (2009) observed an increase in the protein content during the fermentation. The significant increase in protein content during fermentation may be attributed to the efficient bio-conversion of highly polymerized carbohydrates into microbial protein and the production of different types of enzymes, which are proteinaceus in nature (Vijayakumar, 2003; Bhatnagar, 2004).

The crude fat content showed slight increase of about 4.21% to 4.78% on day 9 compared to that of the initial with increase about 13.53%. The increase

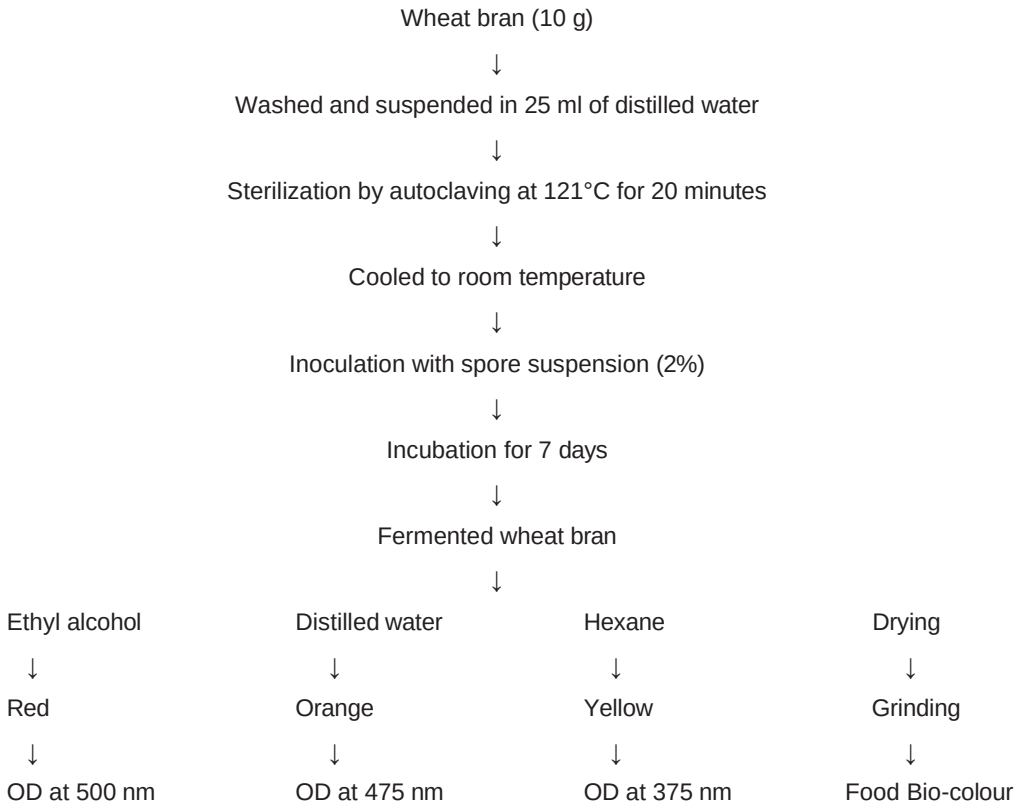

Fig.1. Flow chart for the production of food bio-colours from wheat bran through solid state fermentation

in crude fat content in the fermented wheat bran during SSF till day 9 may be attributed to the production of fungal fatty acids during fermentation as reported by Higashiyama *et al.* (2002). The increase in fiber content was observed from 8.97% to 14.88% contributed to about 65.88% increase after 9 days. It may be attributed to the utilization of easily digestible soluble carbohydrates by the growing fungus, leaving the indigestible fiber content as high as reported by Singh *et al.* (1990).

Crude ash level in fermented wheat bran increased from the initial by about 20.12%, the increase was from 4.92% to 5.91% after 9 days of fermentation. The increase observed in crude ash may be due to the dry matter loss during fermentation causing a relative increase in the unaltered components of the fermented product, especially the fiber and ash contents. The initial pH 6.2 of the wheat bran, decreased slowly throughout the fermentation period of 9 days leads to pH 5.8 at last. The changes in pH of wheat bran content confirmed the metabolism of the fungus.

Solid state fermentation of wheat bran can increases the nutrients availability and improves their sensory characteristics. Thus, there is a value addition to these materials and creating new opportunities for their utilization. This also helps in reducing the environmental pollution concern that their disposal would cause.

Conclusion

The use of wheat bran as a substrate was cost effective and environmental friendly. The results indicated the suitability of wheat bran for production of food bio-colours through solid state fermentation. The good yield of red, orange, yellow and total bio-colour obtained were 126.76 OD Units/g dms at 500 nm, 76.76 OD Units/g dms at 475 nm, 56.05 OD Units/g dms at 375 nm and 259.38 OD Units/g dms respectively with wheat bran medium at optimized process parameters including 75% (w/v) initial moisture content, 0.3-0.4 mm particle size, pH 6, incubation at 30°C, inoculation with 3% of spore suspension of 5 days old culture and an incubation period of 9 days under the influence of fructose (3% w/w) and peptone (1% w/w) as a carbon and nitrogen source respectively.

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