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Article

Use of microalgal biomass as functional ingredient for preparation of cereal based extrudates: impact of processing on amino acid concentrations and colour degradation kinetics

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Suitability of developing Spirulina incorporated cereal based low cost nutritious extrudates was analysed against extrusion processing parameters. Most significant extrusion processing parameters considered for present study were feed moisture (20-25%), die temperature (100-120 °C) and screw speed (50-100 rpm). Different extrusion conditions were used to obtain most acceptable rice: Spirulina blend extrudates. In present study before extrusion processing different additives (citric acid and sodium bicarbonate) were added in rice: Spirulina blend and checked its effect on colour degradation kinetics at varied packaging and storage conditions. Higher screw speed (100 rpm) indicating less residence time of feed material inside the barrel resulted in higher colour retention of rice: Spirulina (97:03) blend extrudates. Kinetics for rice: Spirulina (97:03) blend extrudates indicates faster rate of colour degradation in terms of lightness (half-life of 4 days) when packed in metalized polyethylene at 50°C with 65% relative humidity. Increased concentration of Spirulina (1-3%) in raw formulations resulted in increase in concentration of all amino acids. Impact of extrusion processing has shown non-significant ($p \leq 0.05$) effect on amino acid concentrations of rice: Spirulina blend extrudates. Also, all the spirulina added samples showed good consumer acceptability with the score of 6.7.

Keywords: Extrusion processing. Spirulina. Storage stability. Colour degradation kinetics.

INTRODUCTION

There is wide spread prevalence of nutritional (protein, vitamins and minerals) deficiency in all risk groups especially among children and women throughout the world. Nutrient deficiencies in developing countries are particularly concerning as these countries are home to more than two-third of the world's total population with a high burden of nutrition-related disease. To address these nutritional deficiencies and prevent their sequelae, extrudates can be used as a medium for incorporating nutrients that have functional importance (Camire, 2011). Snack foods products can be of great economic importance to sustainable agriculture in countries having ever-increasing population pressure and facing resource constraints as well as rapid diminution of natural resources. Potential of extrusion cooking technology for the production of snack foods has long been recognised by food processors globally. It is more effective, cleaner and less expensive with a product of the same quality or even better than manufactured with traditional technologies.

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Extrusion cooking being the versatile process combines various unit operations depending on the end use and process conditions used. Nowadays, extrusion plays an important role in several food processing industries due to its versatile nature (Gat, Ananthanarayan, 2015a; Ananthanarayan *et al*., 2018). Although many researches have been undertaken for enrichment of cereal based snacks with incorporation of other ingredients but still no any literature is available on extrusion processing of rice: Spirulina blend extrudates. Cereal based extruded food products often contain less protein content and possess a poor biological value owing to their limited essential amino acid contents and are therefore frequently fortified with proteins (Singh *et al.,* 1991). Microalgae are one of the most promising sources for new food and functional food products, and can be used to enhance the nutritional value of foods, due to their well-balanced chemical composition (Batista *et al*., 2013). Its impressive protein and mineral content attracted the attention of both researchers and industrialists alike. Thus, blends of rice flour and Spirulina powder can be used in combination for development of convenient nutritionally-balanced value added extruded snack foods.

An attempt was made through this study to prepare ready to eat nutritious extrudates. Owe to its green colour Spirulina incorporated ready to eat expanded snack does not require colouring step after extrusion which becomes economically viable for manufactures. Thus, the aim of present study was to evaluate the effect of the extrusion processing parameters on nutritional properties and colour of rice: Spirulina blend extrudates.

MATERIAL AND METHODS

Materials used

Rice (*Oryza sativa)* used as raw material and was purchased from Local market of Mumbai, India and milled to prepare fine flour passing through 80 mesh sieve. Spirulina powder (SP) was purchased from Parchem fine and speciality chemicals, Mumbai, India. Purchased Spirulina has specifications such as fine, uniform powdery appearance of 80 mesh particle size with dark blue-green colour. Packaging materials (polyethylene and co-extruded metalized polyethylene) were procured from Shako Flexi pack Pvt. Ltd., Sion Mumbai, India.

Sample preparation

Rice flour (RF) with Spirulina powder were blended with different proportions (100:0, 99:01, 97:03, 95:05, 93:07) and extruded under fixed extrusion conditions (25% feed moisture, 120°C die temperature and 100 rpm screw speed) to get most acceptable sample. These optimized conditions of feed moisture die temperature and screw speed were selected according to the preliminary (one factor at a time) studies and literature data surveyed (not reported here). After standardizing Spirulina powder concentration (1, 3 and 5%) containing rice extrudate (acceptable sample) were taken for further analysis.

Extrusion cooking

Extrusion cooking was performed in a laboratory scale twin-screw extruder (Brabender GmbH, Germany) as recommended by Gat and Ananthanarayan (2015b). Co-rotating extruder consisted of four heating and cooling zones with barrel length to diameter ratio of 20:1. Effect of various parameters of extrusion process (20-25% feed moisture, 100-120°C die temperature and 50-100rpm screw speed) were checked on colour degradation of rice: Spirulina blend extrudates.

Nutritional analysis

Nutrition analysis such as moisture content (925.10), ash content (method 923.03), protein (960.52) content (Nx6.25), crude lipid (method 922.06) and fiber content (method 962.09) of raw formulations and snack bar was carried out according to AOAC international methods, Horwitz (2000). Carbohydrate content was determined by weight difference method. Raw formulations and extrudates were analyzed for the iron content using atomic absorption spectrophotometer (Model GBC-932 plus) by following the method of Allan (1959). For nutritional analysis all the samples were estimated in triplicates.

Amino acid analysis

The raw formulations and their extrudates were analyzed for the amino acids using a reverse phase HPLC Pico-TAG amino acid analysis system (Waters Corporation, Milford, MA) as described by Paes and Maga (2004).

Colour measurement

Hunter Lab colourimeter (LabScan XE, USA) was used to evaluate colour parameters (L*, a*, b*) of all the rice: Spirulina blend raw formulations and extrudates i.e. before and after extrusion as described by Kaur *et al.* (2017). Reported values of each sample were the average of three replicates. Numerical total colour difference (ΔE) was calculated by following equations.

$$\Delta E = (\Delta L2 + \Delta a2 + \Delta b2)1/2$$

Colour degradation analysis and kinetics

Colour degradation analysis of rice: Spirulina blend extrudates were carried out with or without addition of additives (citric acid or sodium bicarbonate at concentration of 2% each) in polyethylene packaging material at varied temperature and humidity conditions (PE 2565 and PE 5065) as described by Gat and Ananthanarayan (2016).

Zero order kinetic model equation (1) was used to describe colour degradation kinetics of extrudates prepared from rice: Spirulina (97:03) blend.

$$C = C0 \pm k0tC = C0 \pm k0t \tag{1}$$

where *C* is the measure of the value of colour variables at time t, *C0* is the initial value of colour variables at time zero, *t* is the storage time, *k0* represents the zero-order kinetic constant. Half-life ($t_{½}$), the time required for the rice: Spirulina blend to decrease to 50% of its initial colour was calculated from the rate constant as 0.693/k.

Sensory analysis

Sensory analysis were carried out for all dried rice: Spirulina blend extrudate samples as described by Ananthanarayan *et al.* (2017) using a panel of 12 semi-trained panelists (research students and faculty members) from Department of Food Engineering and Technology, Institute of Chemical Technology, Mumbai, India and scores were given with the help of 9 point hedonic scale by considering following criteria: Like extremely: 9; Like moderately: 7-8; like slightly: 5-6; dislike slightly: 3-4; and dislike extremely: 0-2. Overall liking scores given by sensory panelists are mainly dedicated to intensity of colour for the acceptance. Training sessions were held until panel members could identify the same sample that was coded differently in a session. The readings in triplicates were averaged and rounded to the nearest whole number.

Statistical analysis

One-way ANOVA was performed for analysis of experimental values using statistical software (SPSS 16.0). Values were calculated as mean ± standard deviation and significant differences ($p \leq 0.05$) were assessed using Duncan's LSD test. The data of kinetics were analyzed with the help of 'Microsoft Excel' software.

RESULTS AND DISCUSSION

Nutritional properties of raw formulations and rice: Spirulina blend extrudate

In this present study the proximate analysis reported that the rice used contained 12.02% moisture, 0.19% crude fiber, 0.37% crude fat, 0.33% ash and 80.57% total carbohydrates. The amount of protein content of rice sample was estimated to be 6.52% and the addition of Spirulina powder improved it to about 7.56% for raw rice: Spirulina blend (97:03). The enhanced protein content of raw rice: Spirulina blend is due to the inherent high quantity of proteins (63.07%) of Spirulina powder. Belay *et al*. (1993) and Habib *et al*. (2008) also regarded Spirulina as a well-known source of protein with high biological value. The fiber content range of rice alone and Spirulina included rice blend was observed to be from 0.19 to 5.82%. Lowest ash value of 0.33% was observed in case of raw Spirulina. Ash content of raw rice: Spirulina blend improved to about 0.94% with inclusion of Spirulina (**Table I**). Fradique *et al.* (2010) also reported increment in ash content on incorporation of Spirulina in pasta. Remarkable change in the moisture content was observed due to extrusion cooking process. Moisture content of raw rice: Spirulina blend was 10.95% whereas after processing it significantly declined to 8.12% ($p \leq 0.05$). After extrusion cooking process a significant difference in the amount of protein was observed. Protein present in raw rice: Spirulina blend was 7.56% whereas after extrusion cooking it showed a decreasing trend to 7.38% ($p \leq 0.05$). Zhang *et al.* (2017) also reported reduction in soluble protein in extruded samples which might be due to the thermally induced protein aggregation over protein degradation. Rice flour sample showed 0.61mg/100g of iron content and after the incorporation of Spirulina powder it was increased to about 2.26 mg/100g for raw rice: Spirulina blend (97:03). The inherent higher iron content (25.46 mg/100g) of Spirulina powder resulted in increased iron content of raw rice: Spirulina blend (**Table I**). Extrusion cooking showed no significant change in iron content of rice: Spirulina blend. Alonso *et al.* (2011) also reported non-significant changes in mineral composition of pea and kidney bean seeds after extrusion.

TABLE I - Effect of addition of Spirulina on nutritional propertiesof raw formulations and extrudates prepared under extrusion conditions of 25% feed moisture, 120°C die temperature and 100rpm screw speed

	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	Carbohydrates (%)	Iron content (mg/100g)
Rice flour (RF)	12.02±0.02^{a}	0.33±0.02^{e}	6.52±0.06^{d}	0.37±0.03^{c}	0.19±0.03^{d}	80.57	0.61±0.03^{c}
Spirulina powder (SP)	4.43±0.03^{c}	7.5±0.02^{a}	63.07±0.04^{a}	6.20±0.02^{a}	8.50±0.05^{a}	10.3	25.46±0.3^{a}
RF:SP blend (97:03)	10.95±0.03^{b}	0.94±0.02^{c}	7.56±0.02^{b}	0.58±0.05^{b}	5.82±0.02^{b}	75.97	2.26±0.04^{b}
RF extrudate (120°C, 100rpm, 25% MC)	7.01±0.01^{e}	0.41±0.03^{d}	6.63±0.03^{e}	0.33±0.02^{d}	0.17±0.04^{d}	85.62	-
RF:SP blend extrudate (120°C, 100rpm, 25% MC)	8.12±0.03^{d}	1.02±0.01^{b}	7.38±0.05^{c}	0.56±0.01^{b}	5.69±0.03^{c}	78.92	2.28±0.07^{b}

[#]All the values are mean ± SD of fivereplicates. Mean values with different superscripts on the same column differ significantly (Duncan's LSD test, $P < 0.05$).Where, MC is feed moisture content.

Amino acid analysis

Results of **Table II** indicate effect of Spirulina powder addition on amino acid concentration of raw formulations and extrudates prepared from rice: Spirulina blend. Rice along with addition of Spirulina powder increased the concentration of amino acids. Reduction in the quantity of most amino acids was observed after extrusion cooking. Similar findings were reported in case of extruded wheat flour (Bjorck, Asp, Dahlqvist, 1984). Gat and Ananthanarayan (2015b) reported that denaturation of protein due to heat treatment resulted in transamination and deamination reactions and therefore decrease in amino acids. The recommended intake suitable for adults and children, when compared the amino acid of extrudates with FAO and World Health Organization (2006), the values obtained from the extruded blend meets the essential amino acid requirement of adults and also for children in the case of threonine and leucine amino acid.

Lysine was the most affected amino acid amongst essential amino acids which showed losses. This was because of the effect of Maillard reaction (Mauron, 1990), which explored effect of extrusion on lysine. The low moisture and high temperature of extrusion resulted in degradation of starch, which provided reducing sugars (Pham, Del Rosario, 1984) and it modifies the structure of protein, exposing reactive sites, which promotes browning reactions (Mauron, 1990).

Loss of lysine was found in agreement with results observed by various authors in case different cereal-based extruded products. In case of extruded wheat flour by twin screw-extruder, Bjorck *et al.* (1984) showed a loss in lysine of 25 and 37g/Kg respectively at 150 and

200 rpm, at 171°C and 15g/Kg moisture. Also, Bjorck *et al*. (1984) reported a 13g/Kg loss in lysine in case of extruded maize starch biscuit dough in a twin-screw extruder at 170°C/13g/Kg moisture/80rpm. Lasekan *et al*. (1996) reported a 10g/Kg loss in lysine for whole-grain maize flour extruded in a twin-screw extruder containing 45g/Kg feed moisture at 135 ºC temperature. It is worth mentioning the outcome of present study that Spirulina addition leads to a great increase of lysine content in relation to rice (un-extrudate: 2.1 to 3.3%) resulting in improved amino acid content of rice: Spirulina blend.

TABLE II - Effect of Spirulina powder addition on amino acid profile of raw formulations and extrudates prepared from rice:Spirulina blend and compared with RDA for children/adult

Amino acid (g/100g protein)	Rice (100:00)	Rice:Spirulina blend (97:03)		RDA for children	RDA for adult
	Un-extrudate	Un-extrudate	Extrudate		
Histidine	2.03±0.03^{b}	2.22±0.02^{a}	2.10±0.06^{b}	1.90	1.4
Threonine	2.87±0.02^{b}	3.51±0.06^{a}	3.44±0.03^{a}	3.40	2.0
Methionine	0.86±0.04^{b}	1.35±0.05^{a}	1.28±0.02^{a}	2.50	1.9
Valine	4.80±0.03^{c}	5.38±0.03^{a}	5.25±0.02^{b}	3.50	2.4
Phenylalanine	4.31±0.02^{c}	4.50±0.02^{a}	4.42±0.04^{b}	6.30	5.23
Isoleucine	3.58±0.02^{c}	3.77±0.04^{a}	3.68±0.03^{b}	2.80	2.0
Leucine	6.12±0.06^{b}	6.60±0.07^{a}	6.54±0.04^{a}	6.60	4.8
Tryptophan	N/A	N/A	N/A	5.42	5.0
Lysine	2.10±0.02^{c}	3.29±0.05^{a}	2.97±0.02^{b}	5.80	3.4

*All values are mean ± SD of three replicates. Mean values with different superscripts on the same column differ significantly (Duncan's LSD test, P < 0.05). Where, N/A is not analyzed.

Effect of Spirulina concentration for checking degradation of colour of rice: Spirulina blend extrudates

Different concentrations (100:00, 99:01, 97:03, 95:05) of rice: Spirulina were extruded by keeping extrusion processing parameters constant as 25% feed moisture with 120°C die temperature at 100rpm screw speed. **Table III** showed that as the concentration of Spirulina increased (0 to 5%) in raw formulations and rice extrudates, L* and a* values were significantly decreased ($p \leq 0.05$). These observations indicated that before extrusion rice: Spirulina blend formulations were darker and greener with increase in Spirulina concentration. The impact of Spirulina addition on L* and a* values was reduced by extrusion probably due to colour degradation when exposed to high die temperature of extruder. In case of b* value (yellowness-blueness), as the concentration of Spirulina increased (0 to 5%) in raw formulations and rice extrudates, yellow colour was significantly decreased ($p \leq 0.05$). The total colour (ΔE) of Spirulina added rice extrudate was significantly decreased before extrusion as well as after extrusion. Maximum L*, a*, b* and total colour (ΔE) values were reported for control rice extrudate (100:00) with no addition of Spirulina in them.

Sensory scores for various concentration of rice: Spirulina blend (100:00, 99:01, 97:03 and 95:05 found for various concentrated coloured) extrudates were 6.7, 7.3, 8.2, and 7.4 respectively **(Table III)**. It was observed from the results of **Table III** that all the samples got scores above 6.0 which indicated that the samples possess good sensory acceptability. Above 3% addition of Spirulina, the colour of rice extrudates was observed to be of a darker shade which decreased total colour (ΔE) value and hence the overall acceptability. Spirulina extrudates showed significantly higher ($p \leq 0.05$) sensory scores (7.3-8.2) than the control sample (6.7). Sample extruded with 3% Spirulina concentration was found to be most acceptable with acceptable colour and overall acceptability and therefore was further analysed.

Sensory analysis results expressing higher consumer acceptability for Spirulina incorporated extrudates are in agreement with study reported by Malik, Kempana and Paul (2013) for Spirulina incorporated ice-cream resulting higher scores for colour and appearance when compared to control ice cream.

TABLE III - Effect of addition of Spirulina on colour properties of raw formulations and extrudates (i.e before extrusion and after extrusion) prepared under extrusion conditions of 25% feed moisture, 120°C die temperature and 100rpm screw speed

Rice:Spirulina concentration	L*	a*	b*	ΔE	Overall acceptability
Before extrusion					
100:00 (Control)	76.15± 0.02^a	1.89 ± 0.01^a	10.10 ± 0.01^a	76.84 ± 0.00^a	-
99:01	53.38± 0.02^b	-2.45 ± 0.06^b	9.32 ± 0.02^b	54.24± 0.01^b	-
97:03	42.21± 0.03^c	-2.99 ± 0.05^c	8.63 ± 0.03^c	43.19± 0.01^c	-
95:05	39.55 ± 0.01^d	-3.25 ± 0.04^d	8.54± 0.03^d	40.59± 0.03^d	-
After extrusion					
100:00 (Control)	71.24 ± 0.00^a	0.51 ± 0.01^a	7.82 ± 0.02^a	71.49± 0.03^a	6.7 ± 0.13^c
99:01	61.06 ± 0.01^b	-0.59 ± 0.00^b	4.85 ± 0.04^b	61.20± 0.01^b	7.3 ± 0.26^b
97:03	59.07 ± 0.01^c	-1.06 ± 0.04^c	2.57 ± 0.07^c	59.13± 0.05^c	8.2 ± 0.17^a
95:05	46.38 ± 0.02^d	-1.51 ± 0.07^d	1.08± 0.10^d	46.41± 0.03^d	7.4 ± 0.21^b

[#]All the values are mean ± SD of five replicates. Mean values with different superscripts on the same column differ significantly (Duncan's LSD test, $P < 0.05$). Where, L* is lightness; a* is redness-greenness; b* is yellowness-blueness; ΔE is total colour difference.

Effect of extrusion process parameters for checking colour degradation of rice: Spirulina blend extrudates

Effect of extrusion cooking parameters on colour characteristics is indicated in **Table IV**. Hunter Lab colour parameter L* indicates lightness (intensity of colour) and it was observed that with an elevation in temperature, L* value increased significantly ($p \leq 0.05$). It can be concluded that with increase in temperature the colour of the extrudates was degraded. As shown in **Table IV**, 20 to 25% increase in feed moisture content resulted in reduction in the L* value of the extrudates significantly ($p \leq 0.05$). Lightness of rice: Spirulina blend extrudate was significantly affected by speed of the screw due to disparity in residence time of raw material inside the barrel. It was reported from the study that there was enhancement in intensity of green colour of rice: Spirulina (97:03) blend extrudate as L* value reduced significantly ($p \leq 0.05$) while a* value increased with increase in die temperature (100-120°C).

The a* value of rice: Spirulina (97:03) blend extrudate was significantly affected by extrusion processing parameters namely feed moisture and die temperature (**Table IV**). With increase in die temperature and feed moisture, a* value of rice: Spirulina (97:03) blend extrudate increased significantly ($p \leq 0.05$). Decrease in greenness of rice: Spirulina blend extrudates with increase in temperature was might be due to destruction of pigment. As it has been observed with many natural pigments that those are sensitive to thermal processing which limits its usage as colouring component. Greenness of the rice: Spirulina blend extrudate was having more retention with increase in screw speed (50-100rpm) due to decrease in residence time and less contact of pigment with die temperature for a shorter period of time.

Table IV indicated that b* value of rice: Spirulina blend extrudates was significantly affected by extrusion process parameters. Most significant extrusion process parameter which has positive effect was die temperature. Whereas it has been observed that increase in moisture content along with screw speed leads to decrease in b* value. The increase (change in b* value) as an effect of change in extrusion process parameters is due result of products formed by Maillard reactions. Similar result of increase in b* value of paprika oily extract added extrudates were observed with change in extrusion process parameters (increase in temperature and screw speed) due to Maillard reactions product formation (Gat, Ananthanarayan, 2016). From these results it has been observed that the samples with lowest a* value (maximum greenness) possessed lowest b* value (minimum yellowness) and *vice versa*. Hagenimana, Ding and Fang (2006) and Gulati *et al.* (2016) supports our findings.

TABLE IV - Effect of extrusion cooking parameters (feed moisture, barrel temperature and screw speed) on colour properties (L*, a*, b* and ΔE) of extrudates prepared from rice:Spirulina blend (97:03)

Die temp(°C)/ Screw speed (rpm)	Feed moisture 20%				Feed moisture 25%			
	L*	a*	b*	ΔE	L*	a*	b*	ΔE
100/50	55.35±0.01^{c}	-1.14±0.08^{b}	3.44±0.02^{b}	55.46±0.02^{c}	50.55±0.02^{c}	-1.94±0.04^{c}	2.53±0.08^{b}	50.65±0.04^{c}
100/100	52.89±0.02^{d}	-1.59±0.04^{c}	3.01±0.02^{d}	52.99±0.07^{d}	48.00±0.02^{d}	-2.28±0.08^{d}	2.14±0.06^{c}	48.10±0.03^{d}
120/50	65.92±0.05^{a}	-0.39±0.04^{a}	3.49±0.02^{a}	66.01±0.04^{a}	63.26±0.02^{a}	-0.92±0.08^{a}	2.78±0.03^{a}	63.33±0.03^{a}
120/100	61.20±0.03^{b}	-0.41±0.07^{a}	3.25±0.03^{c}	61.29±0.03^{b}	59.07 ± 0.01^{b}	-1.06 ± 0.04^{b}	2.57 ± 0.07^{b}	59.13± 0.05^{b}

[#]All the values are mean ± SD of fivereplicates. Mean values with different superscripts on the same column differ significantly (Duncan's LSD test, $P < 0.05$). Where, L* is lightness; a* is redness-greenness; b* is yellowness-blueness; ΔE is total colour difference.

Kinetics of degradation of colour of rice: Spirulina blend extrudates formulated with additives incorporation

Most acceptable Spirulina powder added rice extrudates (**Figure 1**) were studied for 60 days of storage life at varied storage (25°C and 50°C with 65% relative humidity) and packaging (packed in polyethylene and metalized polyethylene) conditions. Moreover, the effect of addition of additives like sodium bicarbonate (SBC) and citric acid (CA) was studied. Mathematical kinetic modeling for colour degradation of rice: Spirulina blend was undertaken with the help of use of zero order kinetic models (**Figures 2 and 3**). It was reported that the degradation of colour (L* and a*) with or without incorporation of CA and SBC followed a zero-order kinetic model (**Figures 2 and 3**); the statistical values of coefficients of determination R^2 and the estimated kinetic parameters of these models are shown in **Tables V and VI**.

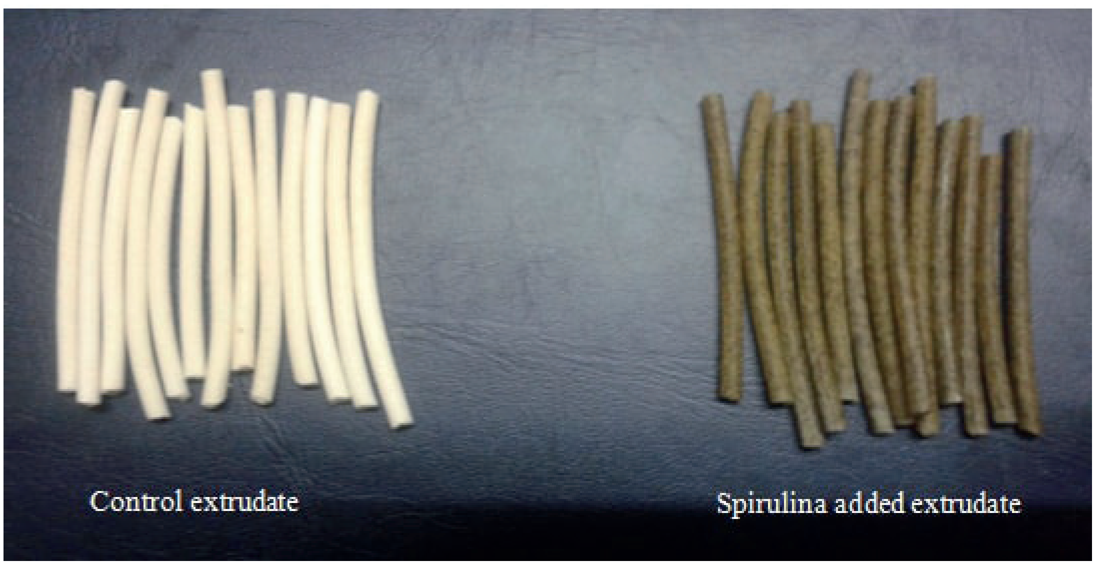

FIGURE 1 - Photographic images of rice extrudate (control i.e. without addition of spirulina) and rice-spirulina (97:03) blend extrudates prepared at 120°C die temperature, 100rpm screw speed and 25% feed moisture.

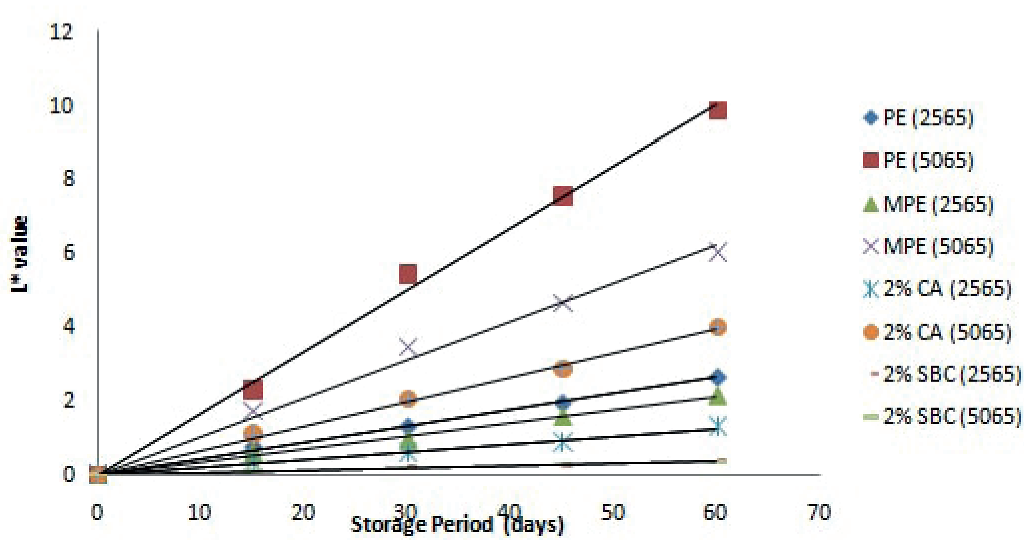

FIGURE 2 - Kinetics of colour (L*) degradation of rice extrudates prepared with and without incorporation of additives (citric acid and sodium bicarbonate at concentration of 2% each) packed and stored in different conditions (25°C and 50°C with 65% relative humidity) as a function of storage time for zero order model.

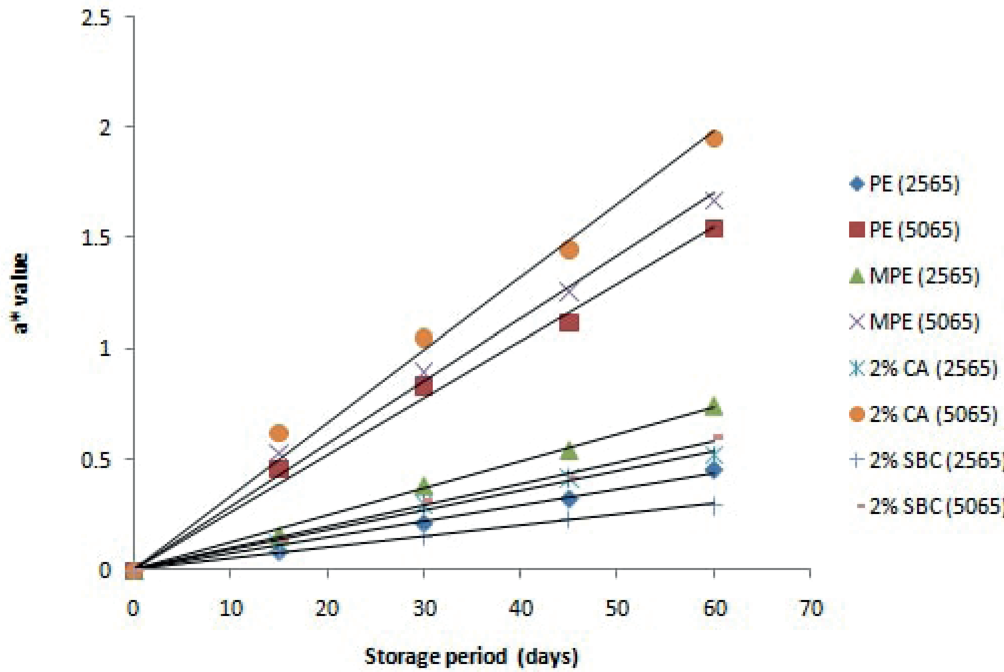

FIGURE 3 - Kinetics of colour (a*) degradation of rice extrudates prepared with and without incorporation of additives (citric acid and sodium bicarbonate at concentration of 2% each) packed and stored in different conditions (25°C and 50°C with 65% relative humidity) as a function of storage time for zero order model.

TABLE V - Rate constant (k), correlation coefficient (R^2) and half-life ($_{t1/2}$) for colour (lightness) degradation of rice:Spirulina blend (97:03) extrudates prepared with/without addition of additives (packed and stored in different conditions).

	Rate constant (k) (time^{-1})	Correlation coefficient (R^2)	Half-life ($t_{1/2}$) (days)	Colour (lightness) degradation (%)
PE (2565)	0.043	0.997	16	9.69
PE (5065)	0.166	0.995	4	36.21
MPE (2565)	0.046	0.993	15	7.65
MPE (5065)	0.103	0.992	7	22.12
2% CA in PE(2565)	0.02	0.992	35	4.37
2% CA in PE (5065)	0.066	0.997	11	12.58
2% SBC in PE (2565)	0.006	0.994	116	1.31
2% SBC in PE (5065)	0.005	0.991	139	1.14

#PE: Polyethylene, MPE: Metalized polyethylene, CA: citric acid, SBC: sodium bicarbonate, 2565: 25°C and 65% relative humidity, 5065: 50°C and 65% relative humidity. #The values of rate constant, correlation coefficient and half life correspond to zero order kinetic fit model. Half-life (t½), the time required for the rice:Spirulina blend to decrease to 50% of its initial colour was calculated from the rate constant as 0.693/k.

TABLE VI - Rate constant (k), correlation coefficient (R2) and half-life (t1/2) for green colour (a*) degradation of rice:spirulina blend (97:03) extrudates prepared with/without addition of additives (packed and stored in different conditions)

	Rate constant (k) (time^{-1})	Correlation coefficient (R^2)	Half-life ($t_{1/2}$) (days)	Green colour (a*) degradation (%)
PE (2565)	0.007	0.992	99	32.7
PE (5065)	0.025	0.992	28	112.61
MPE (2565)	0.012	0.997	58	53.96
MPE (5065)	0.028	0.991	25	122.14
2% CA (2565)	0.009	0.990	77	415.87
2% CA (5065)	0.033	0.990	21	1550.7
2% SBC (2565)	0.005	0.993	139	12.83
2% SBC (5065)	0.009	0.994	77	26.55

#PE: Polyethylene, MPE: Metalized polyethylene, CA: citric acid, SBC: sodium bicarbonate, 2565: 25°C and 65% relative humidity, 5065: 50°C and 65% relative humidity. [#]The values of rate constant, correlation coefficient and half life correspond to zero order kinetic fit model. Half-life (t½), the time required for the rice:Spirulina blend to decrease to 50% of its initial colour was calculated from the rate constant as 0.693/k.

Rice:Spirulina (97:03) blend extrudates packed in polyethylene and stored at 25°C with 65% relative humidity indicated an increase in L* value and decrease in a* value. Similar trend of increase in intensity of colour and greenness was observed for rice: Spirulina (97:03) blend extrudates packed in polyethylene and stored at high temperature conditions (50°C with 65% relative humidity). **Table V** indicates effect of addition of additives on colour degradation of rice: Spirulina (97:03) blend extrudates. From this study it has been observed that rice: Spirulina (97:03) blend extrudates in which no any additives were added are considered as control samples. Theses control samples packed in polyethylene packed indicates maximum (36.21%) increase in the L* value (lightness) with 4 days of half-life. 2% SBC added rice: Spirulina blend extrudate packed in polyethylene showed slight (1.14%) increase in L* value (lightness) indicating maximum colour stability (half-life of 139 days). Storage temperature showed a positive significant effect on L* value for polyethylene and metalized polyethylene packed rice: Spirulina blend extrudates with or without incorporation of additives. Although temperature effect was not much significant in the case of SBC added rice: Spirulina blend extrudate. Rice: Spirulina blend extrudate samples with 60 days storage life at varying packaging and conditions of storage confirmed linear nature indicating increase in percent colour degradation (L* value) to follow kinetics with zero-order reaction. The stability of the sample at extreme conditions (50°C and 65% relative humidity) also shows the possible acceptability of the sample in the extreme regions of India and tropical countries.

CONCLUSION

Physicochemical properties of Spirulina powder added rice extrudates were influenced by processing parameters including feed moisture die temperature and screw speed. Degradation of colour (greenness) of rice: Spirulina (97:03) blend extrudates decreased with decrease in die temperature during extrusion processing. Whereas retention of colour (greenness) of rice: Spirulina (97:03) blend extrudates increased with increase in feed moisture. Incorporation of 2% SBC (as additive) in rice: Spirulina (97:03) blend extrudate resulted in improved colour retention as compare to control sample containing no any additives. Profile of amino acid showed that Spirulina addition leads to a great increase of lysine content in relation to rice (un-extrudate: 2.1 to 3.3%). Outcome of present study indicates the use of Spirulina powder as a natural colourant with increased protein contents in rice: Spirulina (97:03) blend extrudates. Also, present study indicates potential for developing rice: Spirulina blend extrudates with strong commercial nutritional impact.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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REFERENCES

Allan JE. The determination of iron and manganese by atomic absorption. Spectrochim Acta A Mol Biomol Spectrosc. 1959; 1(15):800-6.

Alonso R, Rubio LA, Muzquiz M, Marzo F. The effect of extrusion cooking on mineral bioavailability in pea and kidney bean seed meals. Anim Feed Sci Technol. 2001; 94 (1-2):1-13.

Ananthanarayan L, Gat Y, Kumar V, Panghal A, Kaur N. Extruded black gram flour: Partial substitute for improving quality characteristics of Indian traditional snack. J Ethn Foods. 2017; 5(1):54-59.

Ananthanarayan L, Gat Y, Panghal A, Chhikara N, Sharma P, Kumar V, Singh B. Effect of extrusion on thermal, textural and rheological properties of legume-based snack. J Food Sci Technol. 2018; 55(9):3749-3756.

Batista AP, Gouveia L, Bandarra NM, Franco JM, Raymundo A. Comparison of microalgal biomass profiles as novel functional ingredient for food products. Algal Res. 2013; 2(2):164-73.

Belay A, Ota Y, Miyakawa K, Shimamatsu H. Current knowledge on potential health benefits of Spirulina. J Appl Phycol. 1993; 5(2):235-241.

Bjorck I, Asp NG, Dahlqvist A. Protein nutritional value of extrusion-cooked wheat flours. Food Chem. 1984; 15(3):203-14.

Camire ME. Nutritional changes during extrusion cooking. Adv Food Extrusion Technol. 2011:87-102.

Fradique, M, Batista AP, Nunes MC, GouveiaL, Bandarra NM, Raymundo A. Incorporation of Chlorella vulgaris and Spirulina maxima biomass in pasta products. Part 1: Preparation and evaluation. J Sci Food Agric. 2010:90(10):1656-1664.

Gat Y, Ananthanarayan L. Effect of extrusion process parameters and pregelatinized rice flour on physicochemical properties of ready-to-eat expanded snacks. J Food Sci Technol. 2015a; 52(5):2634-45.

Gat Y, Ananthanarayan L. Physicochemical, phytochemical and nutritional impact of fortified cereal-based extrudate snacks. Nutrafoods. 2015b; 14(3):141-9.

Gat Y, Ananthanarayan L. Use of paprika oily extract as pre-extrusion colouring of rice extrudates: impact of processing and storage on colour stability. J Food Sci Technol. 2016; 53(6):2887-94.

Gulati P, Weier SA, Santra D, Subbiah J, Rose DJ. Effects of feed moisture and extruder screw speed and temperature on physical characteristics and antioxidant activity of extruded proso millet (Panicum miliaceum) flour. Int J Food Sci Technol. 2016; 51(1):114-122.

Habib M, Parvin M, Huntington T, Hasan M. A review on culture, production, and use of Spirulina as food for humans and feeds for domestic animals and fish. FAO Fisheries and Aquaculture. 2008; Circular. No: 1034.

Hagenimana A, Ding X, Fang T. Evaluation of rice flour modified by extrusion cooking. J Cereal Sci. 2006; 43(1):38-46.

Horwitz W. Official methods of analysis of AOAC International. 2000.

Kaur P, Sharma P, Kumar V, Panghal A, Kaur J, Gat Y. Effect of addition of flaxseed flour on phytochemical, physicochemical, nutritional, and textural properties of cookies. J Saudi Soc Agric Sci. https://doi.org/ 10.1016/j.jssas.2017.12.004 (in press).

Lasekan OO, Lasekan W, Idowu MA, Ojo OA. Effect of extrusion cooking conditions on the nutritional value, storage stability and sensory characteristics of a maize-based snack food. J Cereal Sci. 1996; 24(1):79-85.

Malik P, Kempanna C, Aman P. Quality characteristics of ice cream enriched with Spirulina powder. Int J Food Nutr Sci, (2013);2(1):44-50.

Mauron J. Influence of processing on protein quality. J Nutr Sci Vitaminol. 1990;36(Suppl 1):57-69.

Paes MC, Maga J. Effect of extrusion on essential amino acids profile and color of whole-grain flours of quality protein maize (qpm) and normal maize cultivars. Rev Bras Milho Sorgo. 2010;3(1):10-20.

Pham CB, Rosaria RD. Studies on the development of texturized vegetable products by the extrusion process. II. Effects of extrusion variables on the available lysine, total and reducing sugars. Int J Food Sci Technol. 1984;19(5):549-59.

Singh RK, Nielsen SS, Chambers JV, Martinez-Serna AM, Villota R. Selected characteristics of extruded blends of milk protein raffinate or nonfat dry milk with corn flour. J Food Process Pres. 1991;15(4):285-302.

World Health Organization. WHO child growth standards. Training course on child growth assessment. Job-aid: Investigating causes of undernutrition. Geneva: WHO, 2006.

Zhang J, Feng X, Patil H, Tiwari RV, Repka, MA. Coupling 3D printing with hot-melt extrusion to produce controlled-release tablets. Int J Pharma. 2017;519(1-2):186-197.

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