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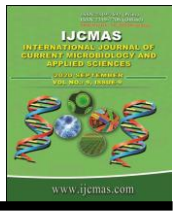

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Process Standardization and Quality Evaluation of Buttermilk and Lemon RTS by adding Red Yeast Rice Powder as Food Colorant

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A B S T R A C T

The present study was carried out to justify acceptability of Red Yeast Rice Powder (RYRP) as a colorant. Addition of 1.0 percent Red Yeast Rice powder to lemon juice and buttermilk was scored highest in terms of color, texture and appearance parameters. The prepared Red yeast Rice powder was stable at room temperature for 90 days when properly packed in suitable packaging material. On the basis of findings, it was concluded that the products prepared by using Red Yeast Rice powder as a colorant could be considered as the best from both nutritional and sensory point of view. It is also economically feasible than synthetic colorants which are being sold in market.

Introduction

India ranks second as a producer and consumer of rice in the world and it contributes for about 22.3% of global production. Red yeast rice (RYR) could be a nutraceutical made by fermentation of polished rice with the mold *Monascus purpureus* and other related species of molds. Red Yeast Rice (RYR) contains various compounds like polyketides, pigments, monacolins, unsaturated fatty acids, and phytosterols. Red Yeast Rice (RYR) has been used as an herbal supplement and in the cooking of East Asian countries like China, Japan, and Korea. Red Yeast Rice has been utilized as an herbal additive and in the cooking of food East Asian countries like China, Japan, and Korea. It has been used for flavoring, coloring, and preservation of food and in traditional Chinese medicine for many years (Burke *et. al.*, 2015).

Red Yeast Rice is the fermented product which is produced from normal rice (*Oryza sativa*) with red mold (*Monascus* spp.). Red

yeast rice is also known with various another name which are ang-kak, hung-chu, hon-chi, hong-gug in Korean, red koji, benikoji, and so on. It has an extended historyas a flavoring, coloring and preservative in food and a folk medicine in many Asian countries. The RYR product is noted to by different names as per the local languages (Woranan and Prasad 2015,). Many species of the mold *Monascus* have also been widely used in making different products like red wine and red soybean cheese (Chen *et. al.*, 1987). (Hesseltine *et. al.*, 1979) The mold *Monascus* first became known in Western world when van Tieghem (1884) enlisted the use of red powder (red yeast rice) by local populations in Java islands. The fungus that was separated from red mold rice was named *Monascus purpureus* Went by scientist (Went, 1895), in recognition of the purple color. Today there are more than 30 *Monascus* strains which are deposited with the American Type Culture Collection (ATCC) or other Institutes like Microbial Type Culture Collection (MTCC), India. Products fermented by *Monascus* (MFPs) are produced by fermentation process with *Monascus* species through solid state fermentation or submerged fermentation processes. The common species of *Monascus* which are usually used for the fermentation are *Monascus purpureus*, *Monascus pilosus*, *Monascus anka* and *Monascus ruber*. The usual *Monascus* yielded products which were consumed over the centuries in Asian countries are Red Mold Rice (RMR), is termed as angkak, anka, and red yeast rice. It has been used from earlier period as food colorant and food preservative, food supplement and in traditional medicine. Scientific studies have confirmed pharmacological effects of *Monascus* fermentate (Endo *et. al.*, 1989) isolated from *Monascus purpureus* a metabolite, monacolin K, which normalized an artificially induced hyperlipoproteinemia in human trials. The reduced form Mevinolin has been introduced as a cholesterol reducing pharmaceutical. Simple extracts of fermentate lower the HDL cholesterol and triglycerides value in blood (Fink *et. al.*, 1989).

Rice (*Oryza sativa L.)* is important food for over more than Thirty percent of India's population, thus holds the key role in food security and plays a very important role in financial setup of national economy. The demand for rice is predicted to grow continuously as population is continuously growing. The rice plant belongs to the monocot genus Oryza of *Poaceae* family. The monocot genus Oryza includes 24 species, of which only two species are widely cultivated like, *Oryza sativa* and *Oryza glaberrima*, are cultivable and the rest 22 species are wild. Rice varieties i.e. sativa is further divided into three subspecies that are, japonica, indica, and javanica. India is that producer of rice varieties which belongs to the subspecies indica Department of Rural Development (DRD 2014). Rice is an annual plant which usually grows to a height of about 0.5–2 m (Gulshan Mahajan *et al.*, 2017).

Red Yeast Rice Powder (RYR) can be used as a colorant to formulate different food products like Ice-cream, Colored sweetened milk, colored lemon juice, colored buttermilk, etc. which improves appearance whereas, Monacolins and HmG co-A reductase have shown reduced risk of cardiovascular diseases and promotes health benefits of Red yeast rice. Thus, it is clear that RYR could be successfully value added. Hence, it can be concluded that the RYR is a potential food supplement prepared from solid state rice fermentation which could be in addition to daily diet. Red Yeast Rice (RYR) can be implemented on commercial scale. RYR contains many compounds including polyketides, MUFA's, phytosterols, pigments, and monacolins (Patel *et. al.*, 2016). Monacolins prohibit activity of enzyme (3-

hydroxy-3-methylglutaryl- coenzyme A) HMG CoA reductase, which limits cholesterol synthesis. There are at the minimum 13 different monacolin compounds have been isolated from RYR, of which some like monacolin K is chemically similar to lovastatin, which is a cholesterol-lowering drug (Zhang, *et al.*, 2016). Ma, 2000 announced that a red mold rice product traditionally manufactured with *Monascus purpureus* had a pigment content of 0.3% in rice flour. Hajjaj, *et al.*, 1999 stated that New food applications, like the coloration of processed meats and other meat products (sausage, ham), marine products like fish paste, surimi, tomato ketchup, yoghurt, wine production, flavored milk, and fruit juices or other fruit products were described.

Materials and Method

Procurement of raw ingredients

Different raw materials that are needed for the preparation of Red yeast rice such as Rice, salt, etc. were purchased from the local market. The culture of *Monascus purpureus* was procured from Microbial Type Culture Collection, Chandigarh (MTCC).

Standardization of procedure for preparation of red yeast rice

Red Yeast Rice is produced by Solid state fermentation of white non sticky rice with *Monascus purpureus*. The *Monascus purpureus* culture is obtained from Microbial Type Culture Collection, Chandigarh (MTCC). To get culture in the vegetative phase the spores were streaked or poured onto sterilized petriplates of PDA (Potato Dextrose Agar). Which were then kept for incubation at 28°C for 5 to 7 days. The Standard culture is obtained from above Mother culture which was then stored under aseptic conditions and Working culture was used for further investigation and studies in the research.

Revival of *Monascus purpureus* Spores

Mother Culture

⇩

Preparation of Potato Dextrose Agar

⇩

Sterilization of PD Agar in autoclave at 121°C for 30 minutes

⇩

Transfer of PD Agar into pre-sterilized petri plates

⇩

Allow agar to solidify in petri plates

⇩

Transfer a loopful of culture from mother culture tube into solidified cooled petri plates

⇩

Keep inoculated petri plates in incubator for 5 to 7 days at 28°C

⇩

Observe for the red colored cottony growth of *Monascus purpureus*

Flowsheet 1: Revival of *Monascus purpureus* spores

Solid state fermentation

Rice variety, that is white and non-sticky after cooking was used in this study. The SSF was performed by following the method explained by scientist (Chairote *et al.*, 2007) with some changes. Rice was soaked in water overnight, after that 50 g of rice were placed in a 250-mlErlenmeyer flask and autoclaved at 121°C for 15 minutes. The moisture content of each rice variety was adjusted to 60% (w/w) on a wet basis. After cooling, 2.5% of the liquid fungal inoculum was added and the inoculated flasks were incubated at 30°C in the darkness. After 14days, the fermented rice was collected and observed for color pigment development and this pigmented rice was dried at 60°C for 4 to 6 hours. In this study three replications were made for rice variety.

Procedure for preparation of red yeast rice

Stepwise production of Red Yeast Rice is carried out by firstly immersion of rice in water for 6 hours following by steaming for 20 min. After that the cooked rice was cooled, from that accurately, 50 g of steam rice was transferred in 250 ml cotton Stoppered Erlenmeyer flask and sterilized at 15 psi pressure and 121°C temperature for 15 min. One week old pure and precultured *Monascus purpureus* was used as inoculums at 2.5% weight basis. The inoculated rice was incubated at 30°C for 14-16 days with constant observations to check the pure growth conditions. The end-product was dried in the oven at temperature not exceeding 65°C for 4 to 6hours to obtain dried red yeast rice. The dried RYR is then powdered using domestic blender (Table 1).

Take 100 gm of rice in 1000ml conical flask

⇩

Wash 3 to 4 times with ample quantity of water

⇩

Prepare 200ml of 0.86 percent saline solution (For this dissolve 0.086gm NaCl in 10ml of water and adjust pH 6.7 with HCl)

⇩

Transfer the solution into conical flask and shake thoroughly

⇩

Place a cotton topper on the neck of conical flask

⇩

Sterilize it in autoclave at 121°C for 25 minutes
(Let the flask cool till it reaches the normal temperature)

⇩

Check Rice grains if they are completely cooked

⇩

To this cooked rice add *Monascus purpureus* culture at 2 percent

⇩

Incubate in Incubator at 28°C for 10-14 days

⇩

Check for color pigment production intermittently

⇩

Transfer the fermented rice from conical flask to the clean and sterilized petri plate and spread evenly

⇩

Dry it in oven at 55°C for 5-6 hours or until moisture content is reduced 10%

⇩

Grinding into powder with uniform particle size and packing in Aluminum foil with LDPE

⇩

Store it in cool and dry place.

Exploration of RYR in Buttermilk

Boil milk and let it cool to warm temperature

⇩

Add Dahi or yoghurt starter, stir it and keep it undisturbed for overnight

⇩

Whisk Dahi with hand blender for 2 minutes

⇩

Add chilled water and let butter separate out

⇩

Add ice cubes till butter firms up

⇩

Remove butter and strain remaining liquid

⇩

Add powdered RYR at given concentrations and mix well

⇩

Buttermilk is ready to be served

Flow Sheet 3: Preparation of Buttermilk (Table 2)

Exploration of RYR in Lemon RTS

Take lemon and squeeze out about 10 ml juice with squeezer.

⇩

Add colored chilled water, sugar and salt to lemon juice.

⇩

Mix well till all of the sugar and salt is dissolved.

⇩

Add 2 to 3 ice cubes and serve chilled.

Flowsheet 4: Preparation of Lemon RTS (Table 3)

Results and Discussion

Physico-chemical properties of selected rice variety

The physical characteristics of Rice play a very important role in development of processing technology. Data from Table 4 showed that the weight of 1000 grains were 18.66 gm, whereas length, width, thickness of flaxseed was 5.41mm, 2.61mm, and 1.83 mm respectively. The shape and color were observed visually, the shape of Rice was found to be slender and color was white.

Chemical composition of rice

From Table 5 it can be seen that the moisture content in Rice was 9.2 percent and carbohydrate content was high and found to be 74.8 percent. The fat content was low at 0.9 percent and protein content was moderate in concentration i.e. 8.4 percent. Ash content of Rice was found to be 0.84 percent.

Effect of cooking on chemical composition of rice

From Table 6 we can see that the nutritional composition of raw and cooked rice varies greatly and found out as Moisture content of cooked rice as increased to 64.04 percent, whereas carbohydrates, fat, and proteins are decreased to 27.8 percent, 0.45 percent, and 2.76 percent respectively. The ash content of cooked rice is negligible at 0.3 percent.

Mineral composition of rice

The concentration of these minerals was recorded to be 80.54, 350.40, and 140.65 (mg/1000g) respectively. The concentration of Calcium, Phosphorus, and Magnesium were much higher than the other inorganic minerals (Table 7).

Exploration of red yeast rice powder as a colorant in buttermilk

The RYR powder concentration in samples is kept at blank for T_0 sample, for T_1 the powder is added at 1.5 percent, for T_2 it was 1.0 percent and for T_3 it was kept at 0.5 percent. The color added was stable for upto15 days in refrigerated conditions with no visual fading or discoloration. Data given in Table 8 revealed that the overall acceptability score recorded for sample T2 was found higher (8.00) followed by T0 (7.38) than other samples. The acceptance of samples depends on the ingredient variation. The overall acceptability among samples was significantly varied statistically. The color and appearance serve as important parameters for the acceptance of food samples.

The highest score for color of complementary juice was recorded for sample T2 (8.0). Whereas, the lowest score received for control sample T0 (7.0). There was a significant difference between the samples in context to color. The maximum score for flavor attribute was received by sample T2 (7.5). While, least score was noted for sample T2 (6.5). The taste of Lemon juice in Table 8 showed that the formulation T2 got the highest value for taste (8.00) and lowest T1 (7.0).

Exploration of red yeast rice powder as a colorant in lemon RTS

The RYR powder concentration in samples is kept at blank for T_0 sample, for T_1 the powder is added at 1.5 percent, for T_2 it was 1.0 percent and for T_3 it was kept at 0.5 percent. Information given in Table 9 explains that the overall acceptability score for sample T2 was found higher (8.25) followed by T3 (7.50) than other samples. The acceptance of samples depends on the ingredient variation. The overall acceptability among samples was significantly varied statistically. The color and appearance serve as important parameters for the acceptance of food samples. The highest score for color of complementary juice was recorded for sample T2 (8.6). Whereas, lowest score was received for control sample T0 (7.0). There was a significant difference between the samples in context to color. The maximum score for flavor attribute was received by sample T2 (7.8). While least score was noted in case of sample T3 (6.5). The taste of Lemon juice in table7 showed that the formulation T2 got the highest value for taste (8.6) against T1 (7.0). There was a significant difference among the samples in context to all the sensory parameters. Overall, by considering the different sensory attributes, the formulation T2 was found to be superior to the other samples (Fig. 1 and 2).

Nutritional content of buttermilk added with RYR powder

Buttermilk is a traditional fermented dairy product (drink). Traditionally it was the clear liquid left after churning butter out of cream. It is a cultured product; however, it is common in warm climates where unrefrigerated milk sours quickly. The product is chosen to give it red color instead of its long known white color which will increase its appearance. The proximate chemical composition of Buttermilk is given in Table 10.

Nutritional content of lemon RTS added with RYR powder

Lemon RTS is consumed by most of the people during the summer season because it provides instant energy and helps in maintaining body fluids. Color plays an important role in acceptability of Lemon RTS. Therefore, it was added with RYR powder which will give red color and thereby induce acceptability. A Typical nutrient composition Lemon RTS is given below in Table 11.

Table.1 Standardized recipe for preparation of red yeast rice powder

Material	Quantity
Rice	100 gm
Salt	0.86 gm
Water	200 ml

Table.2 Recipe for preparation of buttermilk

Ingredients Required	Quantity
Whole Milk	500 ml
Dahi or Yogurt starter	10 ml
Chilled water	500 ml

Table.3 Recipe for lemon RTS preparation

Ingredients Required	Quantity
Fresh Lemon Juice	10 ml
Sugar	15 gm
Salt	0.5 gm
Chilled Water	100 ml

Table.4 Physical properties of rice

Physical Parameters	Observation
Colour	White
Shape	Slender
Length (mm)	5.41
Width (mm)	2.61
Thickness (mm)	1.83
Wt. of 1000 seeds (g)	18.36
Angle of Repose (Degree)	37.1
Density (g/ml)	1.42

Table.5 Chemical properties of rice

Chemical Parameters	Observation (%)
Moisture (%)	9.2
Crude Fat (%)	0.9
Total Carbohydrates (%)	74.8
Total Protein (%)	8.4
Ash (%)	0.84

Table.6 Chemical composition of rice after cooking

Chemical Parameters	Observation (%)
Moisture (%)	64.04
Crude Fat (%)	0.45
Total Carbohydrates (%)	27.8
Total Protein (%)	2.76
Ash (%)	0.3

Table.7 Mineral content in rice

Minerals	Average value (mg/1000g)
Calcium	80.54
Phosphorus	350.40
Magnesium	140.65

Table.8 Sensory evaluation of buttermilk

Treatments		Sensory Characteristics			
	Colour & Appearance	Flavor	Texture	Taste	Overall Acceptability
T_0	7.00	7.00	7.50	8.00	7.38
T_1	7.50	6.50	7.00	7.00	7.00
T_2	**8.00**	**7.50**	**8.00**	**8.50**	**8.00**
T_3	7.50	6.50	7.40	7.60	7.25
C.D.	0.172	0.196	0.196	0.196	0.144
SE	0.058	0.066	0.066	0.066	0.048

Table.9 Sensory evaluation of colored lemon RTS

Treatments		Sensory Characteristics			
	Colour & Appearance	Flavor	Texture	Taste	Overall Acceptability
T_0	7.000	7.500	7.500	7.500	7.380
T_1	7.500	6.500	7.000	7.000	7.000
T_2	**8.600**	**7.800**	**8.000**	**8.600**	**8.250**
T_3	7.500	6.500	7.500	7.500	7.500
C.D.	0.196	0.196	0.156	0.196	0.172
SE	0.066	0.066	0.052	0.066	0.058

Table.10 Buttermilk added with RYR powder

Nutrient	Quantity (per 100 ml)
Energy	29.3 kcal
Carbohydrates	2.3 gm
Proteins	1.7 gm
Fat	1.5 gm

Table.11 Lemon RTS added with RYR powder

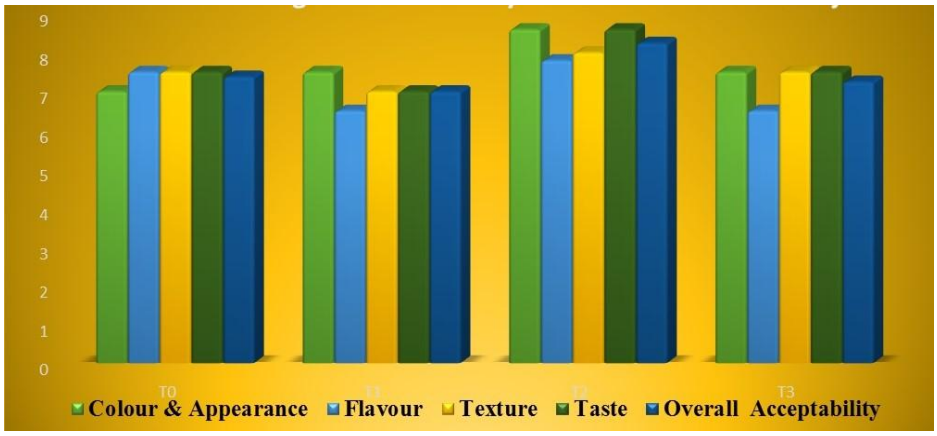
Nutrient	Quantity (per 100 ml)
Energy	48 kcal
Carbohydrates	12.00 gm
Proteins	1.00 gm
Fat	0.00 gm
Vitamin C	53 mg

Table.12 Microbial analysis of red yeast rice powder

Parameter	Observation
Total Plate Count (CFU/mL)	ND
Yeast and Mold Count (CFU/mL)	6.4 x10^8
Coliform Count (MPN/mL)	ND

Fig.1 Sensory evaluation of buttermilk

Fig.2 Sensory evaluation of lemon RTS



Microbial analysis of red yeast rice powder

The growth of harmful and unwanted microorganisms will spoil the prepared product and may lead to different types of food borne diseases which can affect the human health and body. Therefore, microbial analysis of the prepared powder is mandatory to prevent the product from spoilage and also maintain the safety. The data related to microbiological analysis of RYR powder is given in Table 12.

In the present work, the count of beneficial mold was detected as 6.4 x10^8 CFU/ml and bacterial count was not detected in a powder. This count was in suitable range as observed in similar food products. On the other hand, coli-form measure was also carried out. Coliform were not detected in the prepared food sample, which showed that the prepared probiotic

beverage was free of any pathogenic and harmful microbes and safe for consumption.

On the basis of findings, it was concluded that the products prepared by using Red Yeast Rice powder as a colorant could be considered as the best from both nutritional and sensory point of view. The Red Yeast Rice powder at the ratio of 1.0 percent was good in terms of color, texture and appearance. The prepared Red yeast Rice powder was stable at room temperature for 90 days when properly packed in suitable packaging material. The fact that it is cheap than the ones which are being sold in market. Hence, it can be concluded that Red Yeast Rice powder is economically feasible to explore on a commercial scale.

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