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Studies on Exploration of Red Yeast Rice Powder as a Colorant in Ice-Cream and Sweetened Milk

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A B S T R A C T

The present study was carried out to determine acceptability of Red Yeast Rice Powder as a colorant. 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.

Introduction

Rice (*Oryza sativa L.)* is known to be a principal food crop of south and south eastern Countries. It 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).

The word "nutraceutical" and its definition were stated by Stephen DeFelice in year

1989, from the Foundation for Innovation in Medicine (Founder and Chairman), and it recognizes a food or a part of a food, which is of vegetal or animal origin, and features a pharmaceutical activity (DeFelice, 1995). 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).

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 Z. *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 Methods

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) (Table 1–3).

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

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.

Exploration of RYR in Ice-cream

Exploration of RYR in Sweetened Milk

Colored sweetened milk is available in market with different colors like Yellow (Kesar or Badam), Pink (Strawberry or Rose), Chocolate, etc. Therefore, in the Studies of exploration of RYR powder as a colorant colored sweetened milk was chosen as one of the products as it provides great eye appeal and is also a natural colorant, concentration of the powder added is kept at 0.5%, 1.0% and 1.5% of the total milk volume.

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 was5.41mm, 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.

Exploration of red yeast rice powder as a colorant in ice-cream

The RYR powder concentration in samples is kept at blank for T0 sample, for T1 the powder is added at 1.5 percent, for T2 it was 1.0 percent and for T3 it was kept at 0.5 percent. The color added in Ice-cream was stable for up to 45 days in frozen 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 (8.0) followed by T3 (7.5) than other samples. The acceptability of samples varies as the ingredient variation are made.

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 food was recorded for sample T2 (7.6). Whereas, the lowest score received for control sample (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 T1 (6.5). The texture of Ice-cream in table 7 showed that the formulation T2 got the highest value for taste (8.0) 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. 1–3).

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 ice cream

Ingredients Required	Quantity
White Sugar	100 gm
Whipping Cream	100 gm
Milk	1000 ml
CMC Powder	2 gm
GMS Powder	2.5 gm
Vanilla Extract	5 ml

Table.3 Recipe for sweetened milk preparation

Ingredients Required	Quantity
Cold milk	200 ml
Sugar	100 gm
Red Yeast Rice powder	As per formulation
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 ice-cream

Treatments		Sensory Characteristics			
	Colour & Appearance	Flavor	Texture	Taste	Overall Acceptability
T0	7.000	7.500	7.500	7.500	7.400
T1	7.500	6.500	7.000	7.000	7.200
T2	**7.600**	**7.800**	**8.000**	**8.500**	**8.000**
T3	7.500	6.500	7.500	8.000	7.500
C.D.	0.165	0.182	0.156	0.196	0.143
SE	0.056	0.061	0.052	0.066	0.048

Table.9 Sensory evaluation of colored sweetened milk

Treatments		Sensory Characteristics			
	Colour & Appearance	Flavor	Texture	Taste	Overall Acceptability
T0	7.000	7.700	7.500	7.800	7.500
T1	7.500	7.500	7.000	7.000	7.500
T2	**8.600**	**7.800**	**8.000**	**8.500**	**8.250**
T3	7.500	7.500	7.500	7.600	7.550
C.D.	0.167	0.172	0.205	0.182	0.196
SE	0.056	0.058	0.069	0.061	0.066

Table.10 Ice-cream added with RYR powder

Nutrient	Quantity (per 100 ml)
Energy	194 kcal
Carbohydrates	18 gm
Proteins	4 gm
Fat	12 gm
Calcium	110 mg

Table.11 Sweetened Milk added with RYR Powder

Nutrient	Quantity (per 100 ml)
Energy	89 kcal
Carbohydrates	12.0 gm
Proteins	3.0 gm
Fat	3.1 gm
Calcium	120 mg

Table.12 Microbial analysis of Red yeast rice powder

Parameter	Observation
Total Plate Count (CFU/mL)	**ND**
Yeast and Mold Count (CFU/mL)	**6.4×10^8**
Coliform Count (MPN/mL)	**ND**

Fig.1 Red yeast rice powder

Fig.2

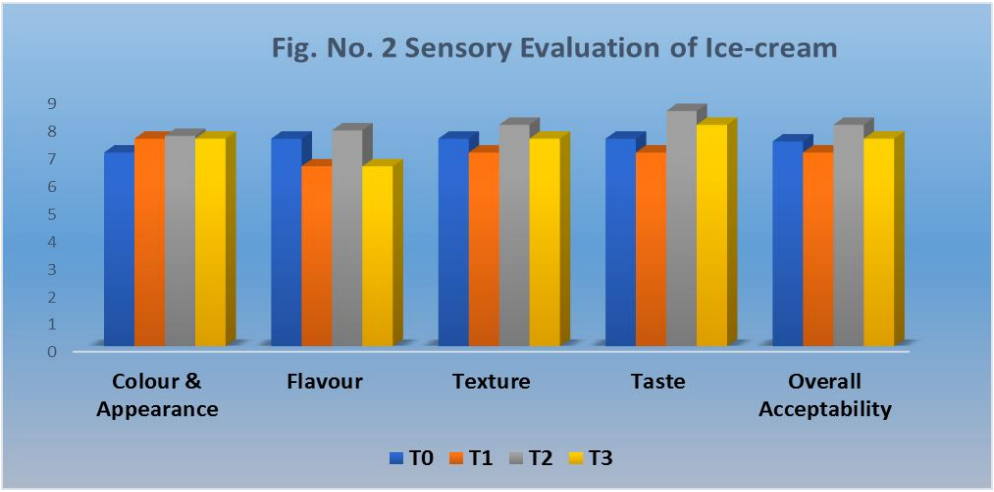
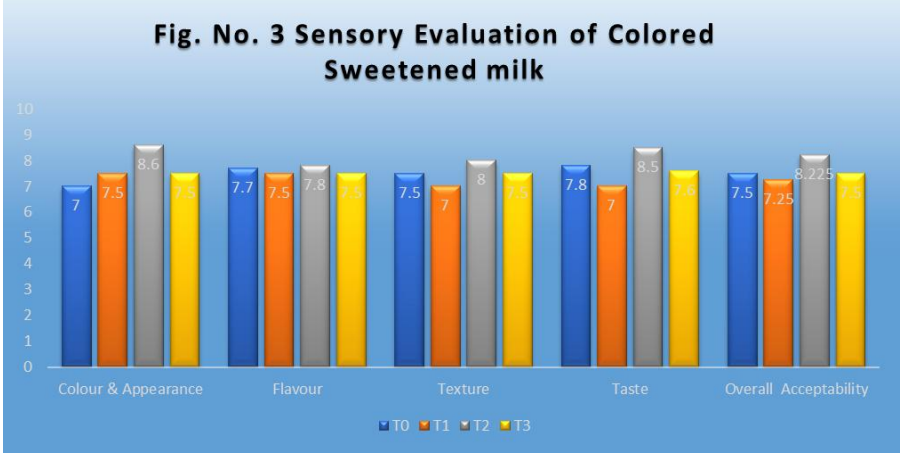


Fig.3



Flow sheet.1 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*

Flow Sheet.2 Production of Red Yeast Rice Powder (RYRP)

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.

Flow Sheet.3 Preparation of ice cream

Add Milk, CMC powder and GMS powder and stir continuously in bowl

⇩

Take Half of the mixture quantity and start heating on low flame, add sugar and stir occasionally. Now allow mixture to reduce to half or till milk consistency is thick.

⇩

Add fresh cream, vanilla essence and powdered RYR and after even mixing is done turn off the flame and transfer the contents into a setting aluminum container with aluminum foil on top and freeze for 6 hours.

⇩

Once the ice cream is set or semi solid blend it in blender to incorporate air

⇩

Transfer again to the container and freeze it for 10 hours

⇩

Vanilla Ice cream with RYR powder incorporation is ready, serve chilled.

Flow sheet.4 Preparation of colored sweetened milk

Add sugar to Water in heavy bottom flask and start heating and stir to dissolve sugar.

⇩

Boil it for 5 minutes on medium flame and add RYR powder according to the recipe to it.

⇩

Let the syrup become slightly thick liquid.

⇩

Cool down syrup and store it in bottle.

⇩

Boil milk and allow it cool for some time.

⇩

Add milk to syrup in 3:1 proportion.

⇩

Stir well and serve chilled.

Exploration of red yeast rice powder as a colorant in sweetened milk

The RYR powder concentration in sample is kept at blank for T0 sample, for T1 the powder is added at 1.5 percent, for T2 it was 1.0 percent and for T3 it was kept at 0.5 percent. The color added was stable for up to 15 days in refrigerated conditions with no visual fading or discoloration in the product color. Data given in Table 9 revealed that the overall acceptability score recorded for sample T2 was found higher (8.25) followed by T3 (7.55) than other samples. The appeal and acceptability of samples depends on variation in the ingredient composition. 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 food was recorded for sample T2 (8.6). Whereas, the lowest score received for the control sample (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 T1 (6.5). The texture of the product from table 9 showed that the formulation T2 got the highest value for taste (8.25) against T1 (7.50). 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.

Nutritional content of ice-cream added with RYR powder

This is near to neutral pH product which is a rich source of fat, milk proteins, and Sugars. Color plays an important role in acceptability of ice-cream. Therefore, we chose it to be added with RYR powder which will give red color and thereby induce acceptability. A Typical nutrient composition or Ice-cream is given below. It can be defined as a soft, sweet frozen food made by milk and cream which is flavored with vanilla, mango, etc. Nutritional information for Ice cream is given in Table 10.

Nutritional content of sweetened milk added with RYR powder

Sweetened flavored and colored milk are available in market to increase the visual appearance and to enhance the color RYR

powder at a concentration of 1% is added to the milk. It is a sweetened dairy drink made with milk, sugar, flavor, and coloring. It is commonly marketed as a pasteurized and chilled product. Nutritional information for sweetened colored milk is given in Table 11.

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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