Shelf-life study of Indian traditional food based nutraceutical (oryzanol) enriched instant mixes Bhath-OZ and Upma-OZ

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Abstract To provide nutraceutical such as oryzanol through food, two instant mixes based on the Indian traditional food cuisine Bisibele bhath and Upma(Bhath-OZ and Upma-OZ) were developed and evaluated for shelf-life. The formulations contained cereals, pulses, and spices along with oryzanol enriched oil and were packed in 200gauge/50 gauge metallized polyester packaging material and stored under ambient (27 °C 65%RH) and accelerated conditions (37 °C/92%RH). Samples were withdrawn periodically and peroxide value (PV), free fatty acid value (FFA), fatty acid composition, oryzanol, and total tocopherols content were estimated. Sensory evaluation of reconstituted products was also carried out. Oryzanol content (610 and 550 mg%) did not change appreciably in Bhath-OZ and Upma-OZ respectively. The peroxide value under ambient condition increased from 1.1 to 9.3 meq.O₂/kg and 2.24 to 9.02 meq.O₂/kg during the 6 month storage study at 27 °C and 65% RH, while under accelerated conditions at 37 °C and 92%RH, it increased from 1.12 to 8.54 meq. O₂/kg and 2.24 to 6.96 meq. O₂/kg during 2 month storage period. Bhath-OZ and Upma-OZ packed in metallized polyester pouches stored at 27 °C and 65% RH had a shelf-life of 4 months without affecting the oryzanol content and quality of instant mixes during the storage period.

Keywords Nutraceutical enriched instant mixes · Oryzanol enriched Bisibele bhath mix · Oryzanol enriched Upma mix · Shelf-life

Rice is one of the oldest food crops in the world. Among the cereals, rice has the highest nutritional quality in terms of digestive energy content of lysine and true digestibility (TD), biological value (BV) and net protein utilization (NPU). Methods employed to improve the nutritional quality of foods, include some of the processing technologies, such as cooking, sprouting, milling and fermentation. However, such processing in the case of cereals is not very common. The regular milled white rice is the rice from which the hull and bran layers have been removed. It is sometimes called milled rice, raw milled rice, milled white rice, polished rice or polished white rice (Knehr 1998). Rice is processed into a number of forms for different value added applications; some cook quickly, withstand harsh processing techniques, or provide texture breakfast cereals (Burrington 2001; Deis 1997). Among the wide variety of rice from which only the outer hull has been removed is known as brown rice. Brown rice is most nutritious form of rice and when cooked and cooled, has slightly chewy texture and nut like flavour (Kuntz 1997), because wax in the outer bran layer reduces the rate of water absorption, and takes longer time to cook than milled white rice (Desikachar et al. 1965). Brown rice has also been reported to lower the risk of diabetes compared to white rice (Mann 2010).

Numerous cereal-based foods are traditionally prepared and consumed all over the world with not much knowledge about their nutritional quality. Scientific knowledge of the beneficial role of various food ingredients for the prevention and treatment of specific diseases has resulted in an increasing number of potential nutritional products with medicinal and health benefits called by different names such as, functional foods, health foods, designer foods, pharma foods etc., Health food is a synonym for the food that can prevent and treat diseases. With these insights, the

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two products namely Bisibele bhath based oryzanol enriched Instant mix (Bhath-OZ) and Upma based oryzanol enriched instant mix (Upma-OZ) were developed, wherein, the products were incorporated with a health improving natural substance, oryzanol concentrate prepared from rice bran oil.

Oryzanol, tocopherols and tocotrienols and the unsaponifiable matter- phytosterols are important minor components of rice bran oil and their beneficial biological effects include cholesterol lowering, anti aging and antioxidant effects (Nicolosi et al. 1992; Seetharamaiah and Chandrasekhara 1989; Gopala Krishna 2002). Such cardioprotective properties cannot be obtained through consumption of brown rice alone. Though brown rice has inherently oryzanol in it to an extent of 50 mg per 100 g, this small amount is not sufficient to provide health benefits. However, at increased levels of oryzanol of 200–2,000 mg, it has been shown to provide the desired health benefits attributed to oryzanol, viz., cholesterol lowering effect in experimental animals (Seetharamaiah and Chandrasekhara 1988). Hence, to provide health benefits, the products, Bhath-OZ and Upma-OZ were developed by incorporating oryzanol, the results of which are presented in this paper.

Brown rice (*Oryza sativa*) was procured from Saraswathi rice mill, Mysore. Red gram dhal (*Cajanus cajan*), green gram dhal (*Phaseolus aureus*), Bengal gram dhal (*Cicer arietinum*), soy chunk (*Glycine max* Merr), fresh onion (*Allium cepa*), green chillies (*Capsicum annuum*), curry leaves (*Murraya koenigii*), spice powder (MTR brand), turmeric powder (*Curcuma domestica*), and table salt (Annapurna brand) were purchased from the local market. Rice bran oil (RBO) was obtained from M/s Habib Agro Industries, Mandya, Karnataka. The oil was enriched with oryzanol concentrate prepared by a patented process. The grains and legumes were cleaned to remove the dust and other extraneous materials, and stored at room temperature (27 ± 2 °C) in plastic containers (100 l capacity). All the spices such as fresh onion (*Allium cepa*), green chillies (*Capsicum annuum*), curry leaves (*Murraya koenigii*), spice powder (MTR), turmeric powder (*Curcuma domestica*), of good quality were purchased from local market.

Processing of brown rice Brown rice was processed under atmospheric pressure and mashed by using a planetary/Hobart mixer for 4–5 min. The mashed material was spread in trays and dried in hot air drier at a temperature of 70–80 °C for 3–4 h. The dried materials were further ground using apex mill (Apex. Community Mill. Apex Construction Ltd., England) to a known particle size of -16mesh.

Legume processing Legumes were hydrated in hot water for 2–3 h and processed using steam under 15–20 lb pressure for 15–20 min and mashed using planetary mixer for 4–5 min. The mashed legumes were dried in a cabinet drier at (70–80 °C) for 3–4 h and further powdered to a particle size of -16mesh.

Bisibele bhath based oryzanol enriched instant mix (Bhath-OZ) Brown rice was pre-processed by using autoclave (Conrad Engelke, Hannover-Limmer) and mashed in a planetary/Hobart mixer for 4–5 min. The mashed materials (rice) were dried in a tray dryer (Precision Products. Vatva, Ahmedabad) for 3–5 h and then powdered. The processed legumes were combined with other ingredients such as extruded soy chunk pieces, dehydrated onion, chilli, and curry leaves. The above ingredients were mixed together followed by addition of spice mix, turmeric powder, common salt and finally with oryzanol enriched rice bran oil. All the above ingredients were mixed together in a ribbon blender (Delight Agencies, Chennai) for 10–15 min to obtain an oryzanol enriched instant mix – Bhath-OZ.

Upma based oryzanol enriched instant mix (Upma-OZ) The product Upma-OZ was formulated in a similar manner to that of Bhath-OZ omitting legumes and spices. All the raw materials were processed under similar conditions as given for the formulation Bhath-OZ. All the other ingredients viz., processed brown rice, extruded soy chunk pieces (*Glycine max* Merr), dehydrated onion (*Allium cepa*), green chilli (*Capsicum annuum*), curry leaves (*Murraya koenigii*), table salt (Annapurna brand) and oryzanol enriched rice bran oil were mixed together in a ribbon blender for 8–10 min to obtain a upma based oryzanol enriched instant mix (Upma-OZ).

Proximate analysis The products were analyzed by standard methods for moisture (AOAC 2000), total fat, crude protein, and ash (AOAC 2000). The carbohydrate content was calculated by difference. Peroxide value (PV) (AOCS method Cd 8–53 1997) and free fatty acids value (FFA), (AOCS method (Ca 5a-40 1997) were determined on the extracted fat and expressed on product basis.

Determination of oryzanol and tocopherols content Oryzanol content of the products were determined as per published procedure on the extracted fat (Gopala Krishna et al. 2001). A known amount of fat (10 mg) was accurately weighed and dissolved in hexane and made upto a known volume and the optical density at 314 nm in a 1-cm cell was measured in a UV-visible double beam recording spectrophotometer (model-UV-1601 Shimadzu Corporation, Kyoto, Japan) followed by calculation using specific

extinction coefficient of 358.9 (Gopala Krishna et al. 2001) and expressed as mg% of oryzanol based on the product. The extracted fat from products was estimated for their tocopherols content after saponification of the fat. The saponified fat was extracted using diethyl ether to get ether extract which after desolventization yielded unsaponifiable matter. Further the unsaponifiable matter was taken in ethanol for colorimetric determination using Emmerie Engel procedure as reported in the vitamin E panel method (Anon 1959) and expressed as mg% of tocopherol in the product.

Fatty acid composition Fatty acid methyl esters were prepared as per the procedure of AOCS O.M.No. Ce 1e-62 (1997) using a gas chromatograph (FISON 8,000 series). Injector port and FID on system were maintained at 240 °C. The column temperature was maintained at 180 °C (isothermal analysis). The GC was equipped with glass capillary column of length 40–60 m with 0.20 to 0.35 µm internal diameter and coated with butanediol polysuccinate (BP2) of coating thickness 0.2 to 0.25 µ. The carrier gas used was nitrogen with a flow rate of 1 ml/min.

Packaging and shelf-life studies For conducting storage (shelf-life) studies, instant mixes Bhath-OZ and Upma-OZ (250 g) having moisture content of about 6.0–7.5% were packed in 200gauge/50 gauge metallised polyester/150 gauge polyethylene pouches (30x25cm). The packs were heat-sealed and stored at 27 °C, (65% RH) simulating ambient condition, and at 38 °C, (92% RH) simulating accelerated conditions for a period of 5–6 months. Then the stored packets in duplicate were withdrawn every month and changes in moisture, fat content, free fatty acids, and peroxide values were estimated. Fatty acid composition of the extracted fat from the product, changes in oryzanol and tocopherol content were estimated periodically.

Sensory analysis Reconstitution of Bhath-OZ: One volume (100 g) of the dry mix of Bhath-OZ was added to 2.5 volumes (150 ml) of boiling water in a cooking pan and stirred. Boiling was continued for 5–10 min under low flame to obtain a ready-to-eat product.

Reconstitution of Upma-OZ One volume (100 g) of the dry mix Upma-OZ, was added to 2.0 volumes (100 ml) of boiling water in a cooking pan and stirred. Boiling was continued for 5–10 min under low flame to obtain a ready to eat product. For both the products after reconstitution, the pan was removed from the flame, covered and allowed to stand for 5 min before sensory evaluation to obtain a homogeneous product. Sensory evaluation was carried out for the prepared products periodically up to 6 months by twenty trained panelists drawn from the different depart-

ments of the institute. Samples were evaluated for five important attributes like appearance/colour, consistency; aroma/flavor, balanced taste and overall quality of the cooked products and mean scores were calculated.

Statistical analysis The products (10 kg each) were prepared in two batches of 5 kg and were mixed together to get an uniform product. The packed product was stored in duplicate in two packaging materials and at two temperatures and analysis carried out in duplicate to have four analytical values. The mean and standard deviation values were calculated and data + standard deviation values presented in tables and in figures. The statistical evaluation by students *t*-test was performed. Based on sensory data for acceptability of the products, the study was stopped once the product became unacceptable.

Proximate analysis The proximate composition of the instant mixes Bhath-OZ and Upma-OZ are shown in Table 1. Except for protein and oryzanol contents, the mixes had more or less similar composition. The protein content (18.0%) was slightly higher for Bhath-OZ than for Upma-OZ (12.0%), and the oryzanol content of the mixes were 610 and 550 mg% respectively. The increase in protein content was due to the use of turdhal which has a high protein content in Bhath-OZ and Upma-OZ doesnot contain turdhal. The total tocopherols content of Bhath-OZ (17 mg%) was slightly higher than that of Upma-OZ (10.1 mg%) probably due to the use of different natural herbs and spices in the product.

Storage study The storage study was planned for 6 months under ambient and accelerated conditions of storage. At the end of each storage period, the withdrawn samples were analysed for moisture content, fat content, peroxide value, free fatty acid value, fatty acid composition, oryzanol and tocopherols content followed by sensory evaluation. Due to spoilage of samples, the study was discontinued after 2 months for samples stored under accelerated conditions and hence values were provided for a period of 2 months only.

Moisture content The products stored under ambient condition (27 °C, 65% RH) showed a slight moisture pick up initially from 6.6% and 7.0% to 7.11 and 8.02% and further it increased slightly at the end of 6 month storage period to 7.6–7.78% at 27 °C which was considered statistically insignificant. The products stored under accelerated conditions (38 °C, 92% RH) showed a higher rate of moisture pick-up due to high humidity. Due to this reason, the samples had spoiled at the end of 2 months storage period and the analysis was stopped at this stage which is shown in Fig. 1(a). A similar observation such as moisture pick up at higher relative humidity has been reported by

Table 1 Chemical composition and sensory quality of instant mixes Bhath-OZ and Upma-OZ

	Bhath-OZ	Upma-OZ
Chemical composition:		
Moisture (%)	6.6±0.21	7.0±0.24
Fat (%)	13.6±0.12	13.4±0.15
Protein (%)	18.0±0.20	12.0±0.11
Carbohydrates (%) (by difference)	60.9	65.0
Free fatty acids value (as% oleic acid/100 g product)	0.39±0.05	0.48±0.05
Peroxide value, (meq. O ₂ /kg product)	0.84±0.1	1.7±0.1
Tocopherols (mg/100 g product)	17.0±1.13	10.1±0.06
Total unsaturated fatty acids (g/100 g product)	10.4±1.04	9.9±1.12
Oryzanol (mg/100 g product)	610±50	550±40
Sensory quality:		
Colour	7.5±0.52	7.5±0.41
Consistency	7.5±0.51	7.5±0.62
Aroma/Flavor	8.0±0.33	8.0±0.63
Balanced taste	8.0±0.74	8.0±0.61
Overall quality	8.0±0.31	8.5±0.22

Each observation is a mean of four determinations (n=4)

other researchers (Tiwari et al. 2009) for rice based snack product. The moisture content of Bhath-OZ and Upma-OZ were not significantly different from the starting value (0.39–1.14% change in units) ($p \leq 0.05$).

Fat content Figure 1(b) shows the fat content of the stored Bhath-OZ(13.44–13.60%) and Upma-OZ(13.40–14.42%), which did not vary appreciably throughout the storage period. This indicates that the product had good storage stability under the conditions employed in the study. The fat content of Bhath-OZ and Upma-OZ were not significantly different (0.48% – 0.68% units) ($p \leq 0.05$).

Peroxide value (PV) and Free Fatty acid value (FFA) The initial free fatty acid content (FFA) was at 0.39 and 0.48% in the case of Bhath-OZ and Upma-OZ respectively. Due to slight pick up in the moisture content (8.02% & 8.72% for Bhath-OZ and Upma-OZ) there was a slight increase in FFA also throughout the storage period. It was found to be 0.39 to 1.14% for BHATH-OZ and 0.48 to 0.68% for UPMA-OZ respectively. The PV value for Bhath-OZ and Upma-OZ which was stored under ambient conditions (27 °C, 65% RH) increased marginally from 1.1 to 9.3 and 2.24 to 9.02 meq.O₂/kg respectively during 6 months storage period. But, under accelerated conditions (38 °C, 92% RH), the PV values increased rapidly from 1.12 to 8.54 and 2.24 to 6.96 meq. O₂/kg at the end of 2 months storage period. In general, the free fatty acids value and peroxide value of processed foods have been reported to increase linearly with the storage period (Tiwari et al. 2009). This has been observed in the present study also. The peroxide value

(2.24 - 9.02 meq. O₂/kg) and free fatty acid value (0.39 – 1.56%) of Bhath-OZ and Upma-OZ were found to increase significantly ($p \leq 0.05$).

Fatty acid composition The% fatty acid composition of the fat extracted from the product Bhath-OZ showed saturated fatty acids, viz., palmitic acid, 18.5±0.21; stearic acid, 2.3±0.20; unsaturated fatty acids, viz., oleic acid, 40.4±1.03; linoleic acid, 35.3±1.04 and linolenic acid, 2.1±0.61. and that of fat from Upma-OZ showed, saturated fatty acids, palmitic acid, 19.5±1.91; stearic acid, 2.4±0.62; unsaturated fatty acids, oleic acid, 41.9±1.02, linoleic acid, 32.5±1.82; and linolenic acid, 1.9±0.84. The fatty acid composition remained unaltered during storage for 6 months at 27° C/ 65% RH indicating good storage stability for the product. There were negligible changes in the fatty acid composition for products stored under accelerated condition during the study period of upto 2 months.

Stability of oryzanol and tocopherols Oryzanol and tocopherols were evaluated periodically from samples stored at both 27°C and 38°C. Both the products showed the same trend in the stability of its minor components during storage. Tocopherol content Fig. 1(e) showed a slight decrease from initial value to final value in both the products stored at 27°C and 38°C, whereas oryzanol content (660 mg/100 g product) did not change appreciably and was almost constant Fig. 1(f) at its initial value till the end of the study. However, minor changes in the content were noticed which is ascribed to the heterogeneous nature of ingredients in the mix with bigger particle sizes such as

Fig. 1 Analysis of parameters of Bisibele bhath and Upma based oryzanol enriched instant mixes Bhath-OZ and Upma-OZ during storage at 27 °C/65% RH and 38 °C/92% RH. **a** Moisture; **b** Fat; **c** Peroxide value; **d** Free fatty acid value; **e** Oryzanol content; **f** Tocopherol content. (Each observation is a mean of four determinations, n=4)

seasoning ingredients and hence is not taken as a major change/loss of this nutraceutical oryzanol during storage of the mixes. Tocopherol and oryzanol contents of Bhath-OZ and Upma-OZ were not significantly different (12–17, 500–580) respectively ($p \leq 0.05$).

Sensory evaluation As shown in Table 1 the reconstituted products which were stored at 27 °C, showed similar quality attributes to that of fresh sample in appearance, consistency, aroma, balanced taste (in terms of spicy, lingering pungency, and harshness) and overall quality at each storage period and up to 4 months. Whereas, the products stored at 38 °C showed a significant reduction in aroma, and colour at the end of 30 days (data not provided). After 60 days, the

products had deteriorated due to the accelerated conditions of temperature and humidity (high temperature and high humidity). Hence, the product was assessed to have a shelf life of 4 months under ambient storage conditions.

From the study it may be concluded that nutraceutical such as oryzanol through food can be provided through the two instant mixes based on the Indian traditional food cuisine Bisibele bhath and Upma (Bhath-OZ and Upma-OZ). Bhath-OZ and Upma-OZ packed in metallized polyester pouches stored at 27 °C and 65% RH had a shelf-life of 4 months without affecting the oryzanol content and quality of instant mixes during the storage period. The oryzanol provided by using the oryzanol concentrate showed cholesterol lowering

effect in experimental animals(data unpublished). Hence, Bhath-OZ and Upma-OZ can be used as health food for improvement of health of the consumers.

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