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Development of ambient storable meal for calamity victims and other targets employing radiation processing and evaluation of its nutritional, organoleptic, and safety parameters

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ABSTRACT

During natural calamities nutritious, safe, and ambient storable meal is issue of immense concern. Recently Joint FAO/IAEA programme of nuclear techniques in Food and Agriculture has undertaken Coordinated Research Project (CRP) where a product 'Stuffed Baked Food (SBF)' was developed in India. SBF consists of partially fermented multigrain dough enriched with 5% saturated fat and stuffed with flour of roasted chick pea; boiled and peeled potato (mashed); and cooked chick pea split (mashed) with spices and salt. Stuffed lobe was convection baked, vacuum packaged and gamma irradiated (15 kGy). The product was well acceptable after prolonged storage at ambient temperature while retaining its quality attributes. SBF can also be useful for other targets like defence personnel, school lunch programme, expeditions, and astronauts. Mutation analyses in models including human TK6 lymphoblast cell line at genes $tk^{+/-}$ and $hprt^{+}$; and bacterial systems [*Escherichia coli* MG1655 cells (*rpoB* gene); and Ames strains (TA 100 and TA 102)] endorsed its genotoxic safety.

1. Introduction

Globally, around 200 million people are annually affected by natural disasters. The availability of nutritious, safe and ambient storable meal during such situations is an earnest need (Leaning, 2014). Unpredictable disasters like earthquakes and flash floods may lead to failure of different logistics and worsen the food supply (Sharma, 2015). Quite often foods served in such situations are unsolicited leading to food borne outbreaks (Kouadio, Aljunid, Kamigaki, Hammad, & Oshitani, 2012; Linscott, 2007; Marx et al., 2006). The Centers for Disease Control and Prevention (USA) has laid guidelines relevant to qualities of such foods like nutritional balance, sensory appeal, low microbial load, no pathogenic contamination, prolonged shelf life (at ambient storage), and suitability for all age groups. The UN World Food Programme (WFP) and Food Bank at Carolina, USA are examples of such initiatives (Seidel, Laquatra, Woods, & Sharrard, 2015; Wetzel & Wallace, 2015). Under WFP, UN in association with USDA distributed foods to the earthquake victims in Nepal (Sharma, 2015). Such foods can also be an effective intervention to other targets including defence personnel, school lunch programme (e.g. mid-day meal), expeditions, and astronauts. For prolonged storage of these ready-to-eat (RTE) foods irradiation can be a very useful technology.

Food irradiation is a well known non-thermal technology having diverse potential to ensure food safety and security while retaining the quality attributes (Diehl, 1995; Roberts, 2014). However, depending upon the packaging environment high radiation dose may negatively affect the sensory quality of the food (Lacroix et al., 2009). Hence, irradiation in combination with other processes can provide a suitable solution (Diehl, 1995). Lack of O_2 in packages has been reported to minimize the radiation induced oxidative changes, storage associated quality deterioration and aerobic bacterial growth during storage (Mantilla et al., 2011).

Joint FAO/IAEA programme of nuclear techniques in Food and Agriculture has undertaken a Coordinated Research Project (CRP-D62009) in 2010 on "Development of Irradiated Foods for Immunocompromised Patients and Other Potential Target Groups" where 16 member states participated including India (IAEA, 2015). The current study deals with the development and optimization of a RTE meal called 'Stuffed Baked Food (SBF)' under this CRP. It is conceptualized on an ethnic product called 'Litti' or 'Bati', a regular food preparation in the disaster prone northern India. Microbiological, nutritional, antioxidant capacity, organoleptic qualities and

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genotoxic safety of the product were evaluated during storage.

2. Materials and methods

2.1. SBF preparation

SBF was prepared as per the optimized process detailed in results and discussion (Fig. S1). Fat enriched product was also prepared by adding saturated fat at optimized concentration (5%) to the fermented dough. Around 1500 packets were prepared, irradiated and stored at ambient temperature (26 ± 2 °C).

2.2. Packaging and irradiation

SBF (3 in no.) were vacuum packed (90%) in laminated low density polyethylene (LDPE) packets and gamma irradiated at different doses (2.5–15 kGy) in a Cobalt-60 based Food Package Irradiator (AECL, Canada; activity 1.97 PBq; dose rate 2.4 kGy/h) at the institute. Dosimetry was performed using cerric-cerrous (15 mM) dosimeter. Dose uniformity ration was 1.25.

2.3. Microbiological analysis

It was performed as per the Bacteriological Analytical Manual (BAM) (US FDA 1988). In brief, 20 g of SBF was homogenized in 100 ml of saline (0.85%) under aseptic conditions and serially diluted in the same. For aerobic plate counts (APC) dilutions were pour plated on plate count agar (PCA) and incubated at 30 °C for 2 days. For spore counts, sample homogenate (1 ml) was heated (80 °C for 10 min) in a water bath, serially diluted, and pour-plated on tryptic soya agar plates for aerobic spore counts (ASC) or on reinforced clostridial agar plates for anaerobic spore counts (AnSC) and incubated at 37 °C for 2 and 4 days in aerobic and anaerobic conditions, respectively. For yeast and mould counts (YMC), homogenate (100 μl) was spread plated on rose-bengal chloramphenicol agar plates and incubated at 25 °C for 5 days. Freshly prepared SBF was also inoculated with *Clostridium sporogenes* (NCIM 2560) spores (1.2×10^4 cfu/g), vacuum (90%) packed, irradiated (15 kGy), and analyzed for AnSC.

2.4. Analysis of physical qualities

Moisture content, water activity, and texture were determined as detailed earlier (Kumar, Khade, Dhokane, Behere, & Sharma, 2007; Kumar, Gautam, Powar, & Sharma, 2010).

2.5. Analysis of nutritional parameters

Sample was ground to powder using mixer grinder for the biochemical analyses.

2.5.1. Macronutrients

Protein content was determined using Kjeldahl method and fat was quantified by gas chromatography (Agilent Technologies GC-FID, CA, USA) (Hajare, Gautam, Nair, & Sharma, 2014). Carbohydrate content was determined by deducting the percentage values of moisture, ash, protein, and fat from total (100%). Energy was determined based on protein, sugar, fat and carbohydrate contents.

2.5.2. Micronutrients

For vitamin B1 estimation, sample (1.5 g) was suspended in 0.1 N HCl (50 ml) and boiled for 45 min. Sodium acetate (2.5 N, 5 ml) was added, volume adjusted to 100 ml with distilled water and centrifuged (3000×g for 30 min at 4 °C). An aliquot (5 ml) of the supernatant was mixed with equal volume of alkaline potassium ferricyanide [0.03% of stock prepared in NaOH solution (15.2%)], isobutyl alcohol (20 ml), and ethanol (1 ml) and fluorescence was measured (Hashmi, 1973). The content (mg/100 g) was calculated as:

$$\frac{\text{E (fluorescence of sample solution)} \times 0.04}{\text{Wt (weight of sample)}}$$

For vitamin B2 estimation, sample (1.5 g) was suspended in 0.1 N HCl (50 ml), heated at 121 °C under pressure for 30 min and after cooling pH was adjusted to 6.5 using sodium hydroxide (1 N). HCl (1 N) was added to reduce the pH to 4.5 and volume adjusted to 100 ml with distilled water. The supernatant was filtered (Whatman no.5) and fluorescence was measured before and after adding sodium hydrosulphite (Hashmi, 1973). The content (mg/100 g) was calculated as:

$$\frac{\text{E (fluorescence of sample solution)} \times 0.07}{\text{Wt (weight of sample)}}$$

Vitamin B3 (niacin) estimation relies upon its reaction with cyanogen bromide forming pyridinium compounds derivatives which couples with aromatic amines to produce colored compound (BIS-IS 5400, 1969). Sample (2.5 g) was suspended in distilled water (90 ml), calcium hydroxide (1.5 g) was added, heated for 2 h in Erlenmeyer flask at 121 °C under pressure, and later cooled to 40 °C. The volume was adjusted to 100 ml with distilled water. Later, 50 ml of this suspension was placed on ice bath for 15 min, centrifuged and supernatant (20 ml) was transferred in another centrifuge tube containing ammonium sulfate (8 g) and 2 ml of phosphate buffer (1.12 M, pH 7.1). The suspension was heated at 60 °C, centrifuged, and filtered. For colour development, 5 ml of the filtrate was mixed with cyanogen bromide (10%; 10 ml) and 1 ml of sulphanilic acid (55%; pH 7), placed on ice bath and the absorbance at 470 nm was measured at 100% transmittance using a colorimeter. Similarly, curve was plotted for niacin standard. Reagent and sample blank readings were also determined and niacin content was calculated as:

$$\text{Niacin (μg)/100 g} = K \times 8 \times 100$$

where, K = concentration of niacin derived from standard curve corresponding to A_{470} of sample − A_{470} of reagent blank.

Vitamin A and total vitamin C were also determined as detailed earlier (Hajare et al., 2014). Iron and calcium were determined by atomic absorption spectrometry.

2.6. Antioxidant capacity

1,1-diphenyl-2-picryl-hydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical-scavenging assays, and ferric reducing antioxidant potential (FRAP) were determined as described earlier using ascorbic acid (5–50 μM) as standard (Arnao, Cano, & Acosta, 2001; Kumar et al. 2010).

2.7. Organoleptic analysis

The organoleptic analysis was performed on a 9 point hedonic scale based on appearance, color, texture, aroma, and taste in a Taste Panel Laboratory in individually partitioned compartments (Kumar et al., 2007). The panelists (40 no.) involved included institutional colleagues, defense personnel and students from various universities.