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Effect of Extrusion Technique on Microbiological Quality Assessment of Composite Flour Ready to Eat (RTE) Foods

Harini Venugopal., Jayaprakasha, H.M and Arun kumar, H
Dairy Science College, KVAFSU, Hebbal, Bengaluru-560024, INDIA

----------ABSTRACT----------
Composite flour based RTE food was formulated with Nutricereals (Finger millet, Foxtail millet, Little Millet) along with some of the functional ingredients such as whey protein concentrate and horse gram along with added sugar and other herbal ingredients. Composite flour: Corn-wheat (75: 25), 20 per cent sugar, 10 per cent horse gram and 8.0 per cent WPC comprised sweetened composite flour RTE food and 5% spice mix replacing sugar comprised spiced based composite flour RTE food respectively. Extruded products such as snack foods and breakfast cereals are considered microbiologically safe to eat because the raw materials are subjected to high temperatures (higher than 130°C) and the water activity of the product is between 0.1 and 0.4 because the product is dried to a moisture content of less than five per cent . The formulated composite flour based RTE food was developed using single screw extruder ; both sweetened and spiced RTE food were subjected to various shelf stability tests at different proportions of CO_2: N_2 mixtures. The product packaged with metalized polyester under MAP with 20: 80 gas mixtures was found to be most deserving and highly acceptable combinations resulting in eliminating a high amount of micro organisms with shelf stability of 105 and 120 days for sweetened and spiced composite flour RTE foods respectively at ambient temperature.

Key Words: *Minor millet, Finger millet, Foxtail millet, Little Millet, Horse gram, WPC, Composite flour, RTE food*

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I. INTRODUCTION

RTE foods facilitate independence because of their ease of preparation which means that children and adolescents can be responsible for their own breakfast or snacks. Such foods may need to be reconstituted, pre-heated in a vessel or allowed to thaw if frozen before consumption, or they may be eaten directly without further treatment. Their consumption has also been extended to non-breakfast hours and often serve as in-between meal as well.

Extrusion-cooking is increasing popularity in the global agro-food processing industry, particularly in the food and feed sectors to produce RTE foods. Food extrusion is a thermo mechanical processing operation that combines several unit operations such as mixing, kneading, shearing, conveying, heating, cooling, forming, partial drying or puffing. Food materials are plasticized and cooked in a minute by a combination of moisture, pressure, temperature and mechanical shear, resulting in molecular transformation and chemical reactions. It reduces the microbial count and inactivates the enzymes. It is a multi-step, multi-function thermal or mechanical process, has permitted a large number of food applications. Beneficial changes in the bioavailability as well as in the content of nutrients may take place during extrusion. It is being used increasingly in the food industries for the development of new products such as cereal based snacks, including dietary fiber, baby foods, breakfast cereals and modified starch from cereals (Navale *et al*., 2015; Adekola, 2016). Extrusion offers many advantages over traditional food unit operations such as minimizing time, energy, and cost inputs while adding versatility and edibility to the manufacturing process. A wide variety of different products can be produced by changing ingredients, operating conditions, and/or minor components of the extruder. (Chakraborty *et al.,* 2009).

Effects of extrusion cooking on nutritional quality are ambiguous. This technology because of its beneficial effects such as destruction of antinutritional factors, increased soluble dietary fibres, reduction of lipid oxidation and contaminating microorganisms plays an important role in the production of a wide variety of foods and ingredients (Singh *et al*.,2007; Nikmaram *et al*., 2015).

Spices are an important bio nutrient for both food ingredients and nutritional supplements From ancient times, spices have been used as food additives to enhance the taste and flavor of food. Apart from these spices also have numerous medicinal properties and used to treat several disorders that form an important part of the Ayurvedic Pharmacopoeia (Indian System of Medicine). Spices have increasingly larger role to play in Indian recipes as the bactericidal, bacteriostatic, fungistatic, antifertility, antihelminthic and other medicinal properties and also believed to aid digestion

Methanolic extract of coriander was analysed for presence of antioxidants. The result revealed that polyphenols including gallic acid, caffeicacid, ellagicacid, quercetin and kaemferol are principle component responsible for antioxidant property of coriander leaves (Anitha *et al*., 2014). Mint extract investigated for its antibacterial activity against seven selected pathogenic bacteria: *Bacillus fastidiosus,Staphylococcus aureus, Proteus mirabilis, Proteus vulgaris, Salmonella choleraesuis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Serratia odorifera.* (Kodandaramreddy and Kavita, 2013).

Cuminseed oil and alcoholic extract inhibited the growth of *Klebsiellapneumonia*.Cumin has also found the biofilm-formation preventive properties against *Streptococcus mutans* and *Streptococcus pyogenes*. It has shown the anti-fungal activity against food, soil, animal and human pathogens, yeasts, aflatoxins and mycotoxin producer. The essential oil of was active against *Aspergillus niger, Bacillus subtilis, Staphylococcus epidermidis, Saccharomyces cerevisiae* and *Candida albicans* (De *et al.*,2009).

The green chillies possess the antioxidant phytochemicals, polyphenols deserve a special mention due to their free radical scavenging properties. Antioxidant compounds and their antioxidant activity in 4 different colored (green, yellow, orange, and red) were investigated. Antioxidants present in the (*Capsicum annuum* L.) are β-carotene (5.4 μg/g), capsanthin (8.0 μg/g), quercetin (34.0 μg/g), and luteolin (11.0 μg/g). They protect the food or body from oxidative damage induced by free radicals and reactive oxygen (Nadeem *et al*., 2011). Garlic preparations have been shown to exhibit antibacterial activity against *Helicobacter pylori, Shigella dysenteriae, Shigella flexneri, Shigella sonnei and Escherichia coli* (Ahmed *et al*., 2015). Ginger has strong antibacterial and to some extent antifungal properties. In vitro studies have shown that active constituents of ginger inhibit multiplication of colon bacteria. These bacteria ferment undigested carbohydrates causing flatulence. This can be counteracted with ginger. It inhibits the growth of *Escherichia coli, Proteus sp, Staphylococci, Streptococci* and *Salmonella* (Zadeh and Kor, 2014*)*

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Effect of Extrusion on Micro-organisms

The extruded products such as snack foods and breakfast cereals are considered microbiologically safe to eat because the raw materials are subjected to high temperatures (higher than 130°C) and the water activity of the product is between 0.1 and 0.4 (Fellows,2000) because the product is dried to a moisture content of less than five per cent and eliminates a high amount of microorganisms (Baik *et al*., 2004).The heat-resistant microorganisms showing that shear stress may be involved in microbial load reduction during the extrusion process, predicting that mechanical forces might cause cell rupture of *Bacillus stearothermophilus* (Fraiha *et al.*,2011). In extrusion cooking thermal processing is designed to eliminate mesophilic organisms. Most pathogenic organisms in feed would be inactivated by extrusion cooking through selecting extruder conditions (Okelo *et al*., 2006).

II. MATERIALS AND METHODS

2.1 Process optimization for the preparation of composite flour sweetened RTE food

Malted finger millet, foxtail millet, little millet was blended at 1:1:1 ratio with 20 per cent sugar.The blended nutricereal based flour was used to replace corn-wheat flour at the rate of 25, 50,75 and 100 per cent levels. According to physioc-chemical, rheological and sensory attributes Composite flour: Corn-wheat (75: 25), 20 per cent sugar, 10 per cent horse gram and 8.0 per cent WPC comprised sweetened composite flour RTE food The millet based spiced RTE food based on composite flour was formulated using the composite flour mixture of finger millet, foxtail millet and little millet with corn-wheat (75:25), 20 per cent horse gram and 8 per cent WPC. To these formulations spice mix (containing mint, coriander, garlic, ginger and jeera) was added at the rate of 5, 7 and 9 per cent and subjected to extrusion process followed by oil frying. Such developed product was subjected to rhelogical and sensory tests. The result revealed that addition of 7 per cent spice mix to the composite formulations was highly suitable (Harini *et al* 2019)

2.2 Microbiological Analysis

Microbiological media such as Standard Plate Count Agar (SPCA), Violet Red Bile Agar (VRBA), Malt Extract Agar (MEA) was procured from HiMedia to enumerate total bacterial count, coliforms, yeast and molds respectively in the formulated products.

Total bacterial count

The total bacterial count was estimated on plate count agar (PCA) medium as per the procedure given by IS: 1224 part I and Part II, 1981.

Coliform count

The coliform count was estimated on violet red blue agar (VRBA) medium as per the procedure given by IS: 1224 part I and Part II, 1981.

Yeast and mold count

The yeast and mold count was determined using malt extract agar (MEA) medium as per the procedure given by IS: 1224 part I and Part II, 1981.

2.3 Microbiological Storage stability of formulated Nutricereals based RTE foods

The best adjudged formulations of Nutricereals based RTE food was packaged in Metalized polyester packaging material under normal and modified atmospheric packaging conditions along with the control. The gases used in the study for MAP were carbon dioxide and nitrogen at the ratio of 20:80, 30:70, 40:60 and 50:50. The packaged products were stored at room temperature (30^oC) for a period of four months. At a regular interval of 15 days the samples were analyzed for various microbiological assessesment in order to elicit the effect of MAP. The microbial load with respect to TBC, coliforms; yeast and molds were analyzed at an interval of 15days to adjudge the storage stability of nutricereal based RTE foods.

The millet based composite flour RTE food developed was subjected to storage stability test. Based on the result obtained, RTE food both sweetened and spiced were subjected to MAP. Among different levels of gas mixtures 20: 80 (CO_2: N_2) exhibited excellent storage stability. The selected sample along with the control was further analyzed for microbial quality with respect to total bacterial count, Coliform count ,Yeast and mold count

III. RESULTS AND DISCUSSION

The effect of MAP on microbial quality of millet based composite flour RTE food is presented in Table .The results revealed that the control sample had 2.80 to 4.15 $\log_{10}$ cfu/g of microbial load from the zero days to 60 days of storage period. The coliforms and yeast & mold were found on 60^{th} day of storage with 1.00 and 1.00 $\log_{10}$ cfu/g respectively. Whereas the sweetened RTE food resulted in 1.18 to 2.30 $\log_{10}$ cfu/g of microbial load from 0^{th} day to 120^{th} days.

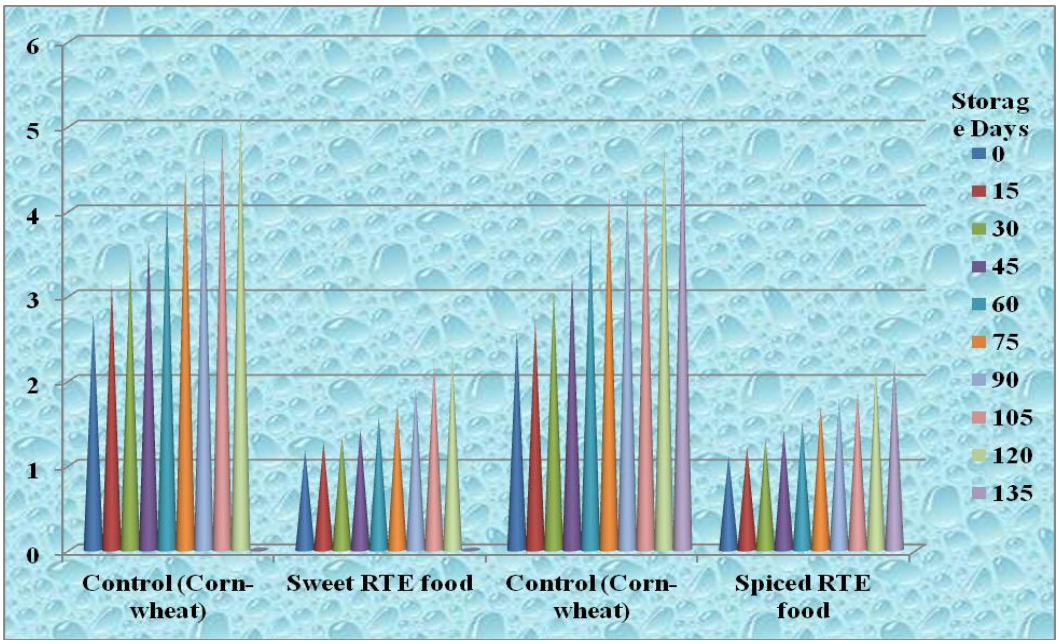
Similar pattern was also observed for the spiced RTE food based on composite flour. As per observations, as the days of storage increased the microbial load also increased considerably but results were within limits. The control product had 2.58 to 4.21 $\log_{10}$ cfu/g from 0^{th} to 75^{th} days of storage and spiced RTE food resulted in 1.11 to 2.25 $\log_{10}$ cfu/g from 0^{th} to 135^{th} days of storage. on the other hand, coliforms and yeast & mold was found on 75^{th} day of storage with 1.20 and 1.10 $\log_{10}$ cfu/g respectively. The result show cases that the formulated RTE food using composite flour had better storage stability when compared to control. It was observed that sweetened RTE food had shelf stability of 105 days and spiced RTE food had 120 days of shelf stability under normal conditions of storage when Packaged in metalized polyester using 20: 80 gas mixture (CO_2: N_2) sounded stable throughout the storage period at ambient temperature.

The analysis revealed that there was progressive increase in the counts for control. However, sweetened RTE food had 2.20 log cfu/g on 105^{th} day of storage and corresponds of counts for spiced RTE food had 2.25 log cfu/g on 120^{th} day of storage period. Indicating the minimum level of total bacterial count. coliforms, yeast and molds were found to be nil for both the products. Thus, emphasizing the composite flour RTE food for safe consumption and storage. The heat-resistant microorganisms showing that shear stress may be involved in microbial load reduction during the extrusion process, predicting that mechanical forces might cause cell rupture of *Bacillus stearothermophilus* (Fraiha *et al.*, 2011). In extrusion cooking thermal processing is designed to eliminate mesophilic organisms. Most pathogenic organisms in feed would be inactivated by extrusion cooking through selecting extruder conditions (Okelo *et al*., 2006).

On the other hand, the utilization of spice mix in the formulation of RTE food may be inhibitory to common pathogenic bacteria such as E. *coli*, *Bacillus subtilis*, *Salmonella spp* and *Bacillus cereus.* Moreover, pathogens exhibit varied responses to water activity in foods. Their growth, sporulation, germination, morphology is controlled by water activity. At reduced water activity, a great decrease in growth occurs dried products (Jayaprakasha *et al*., 1997 and Deviliehere *et al*., 2014). Also, the presence of polyphenol, gallic acid and quercitin components found in millets exhibits antimicrobial effect leading to inactivation of microbial enzymes causing lysis. (Palaniswamy *et al*., 2011).

The formulated RTE food subjected for microbiological enumeration revealed that the coliforms, yeast and molds were found to be nil. The total bacterial count was least as the RTE food undergoes various processing parameters during formulation and extrusion process, keeping the total count to minimum level. on the other hand, spices exhibit anti-bacterial activity, indicating that sweetened and spiced RTE food based on composite flour had a storage stability of 105 and 120 days respectively at 30 ± 1°C when packaged in metalized polyester under MAP with 20: 80 ratios of CO_2: N_2. Thereby the results confer that RTE product having minimum microbial growth, with low water activity, packaged in metalized polyester with MAP has tremendous shelf stability. The results obtained in the investigation are in conformity with the findings of Church and parsons (1995), Mukhtar and Ghori (2012), Indu *et al*. (2014) and Ahmed *et al.* (2015).

Figure 1 : Single screw extruder used for Research



Note:
X Axis Types of Ready to Eat (RTE) Foods
Y Axis Total bacterial count $\log_{10}$ cfu/g

Figure 2: Effect of Modified Atmosphere Packaging (MAP) on microbiological quality of millet based composite flour RTE food

Table 1: Effect of Modified Atmosphere Packaging (MAP) on microbiological quality of millet based composite flour RTE food

Storage period (Days)											
$\log_{10}$ cfu /g											
Type of RTE food	**Type of microbial count**	**0**	**15**	**30**	**45**	**60**	**75**	**90**	**105**	**120**	**135**
Control (Corn-wheat)	Total Bacterial count	2.8	3.15	3.43	3.68	4.15	-	-	-	-	-
	Coliforms	-	-	-	-	1.00	-	-	-	-	-
	Yeast and molds	-	-	-	-	1.00	-	-	-	-	-
Composite flour based Sweetened RTE food	Total Bacterial count	1.18	1.27	1.35	1.43	1.58	1.72	1.92	2.2	2.3	-
	Coliforms	**Nil**									
	Yeast and molds	**Nil**									
Control (Corn-wheat)	Total Bacterial count	2.58	2.71	3.1	3.28	3.78	4.21	-	-	-	-
	Coliforms	-	-	-	-	-	1.20	-	-	-	-
	Yeast and molds	-	-	-	-	-	1.10	-	-	-	-
Composite flour based Spiced RTE food	Total Bacterial count	1.11	1.21	1.31	1.41	1.51	1.7	1.8	1.88	2.15	2.25
	Coliforms	**Nil**									
	Yeast and molds	**Nil**									

Note: * All values are average of three trails

IV. CONCLUSION

The effect of extrusion processing with improved functional properties of extruded snack foods are developed from cereal and legumes fortified with herbs, vegetables dairy and nondairy ingredients. Value addition and formulation of millet-based foods help not only in creating better avenues for utilization and may also help in development of health rich products. Food industries are focusing energies towards the development of functional foods and food ingredients. Processing of millets to incorporate them in ready-to-eat foods can increase their nutritional value, availability and economic value besides improving farmers income and ease of commercialization with extended shelf life.

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