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Research Paper

Effect of Packaging Materials and Storage Conditions on Bio chemical and Microbial profile of Mung Bean *Warrian* (Indigenous Fermented Food)

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Abstract

Mung bean *warrian* were prepared by blending black gram flour with mung bean flour. These are relished equally along with other types of warrian around the world export potential of these products has been marred by microbial spoilage during transportation and storage. The growth of proteolytic bacteria, effect of packaging materials and storage conditions were studied in mung bean flour blended warrian over the year and compared with control traditional black gram flour *warrian*. The results revealed more biochemical degradation of the product in the third quarter of the year in (rainy, hot and humid weather) when proteolytic count was the highest. As compared to the control samples, the pH (4.2), reducing sugar (8.5 mg g^{-1}) and total proteins (22.4 %) were reduced highly in the third quarter while values for total solids (18.5%), soluble proteins (35.5 mg g^{-1}), free amino acids (45.2 ìg g^{-1}) and proteinase activity (7.4 IU g^{-1}) were increased. Out of the different packaging materials (paper, low density polythene (LDPE), tin containers, glass containers, aluminium laminates, and PET (Polyethylene terephthalate) jars employed Aluminium (Al) laminates (0.035 – 0.096%) and PET jars showed negligible moisture pick up (0.033 – 0.091%) from the environment while the paper packages picked up the highest moisture (8.2 – 13.9%). The proteolytic count showed similar trend with moisture pick up, highest in paper packages (4.25 – 4.75 Log_{10} CFU) and lowest in Al laminates and PET jars (3.90 – 3.95 Log_{10} CFU).The study showed that proteolytic bacteria in mung bean flour blended *warrian* grow better in hot and humid conditions resulting in the flavor changes in the product. PET jars and Aluminium laminates were suitable the best packaging and storage material for mung bean flour blended *warrian*.

Keywords: *Warrian*, Mung bean , proteolytic bacteria, moisture, fermented food, Packaging

Introduction

Mung Bean Warrian are cereal-based popular fermented food made from black gram flour blended with mung bean flour along with seasonings. These are sun-dried dumplings, spicy, hollow and brittle friable balls which are used as a condiment or as an adjunct for cooking vegetables, grain legumes or rice. These products are prepared by natural fermentation with traditional methods, but now modern mechanization and microbial technology is replacing these methods (Aidoo *et al.*, 2006). *Warrian* making was traditionally confined to the household, but in recent years, it has acquired the status of a cottage and small-scale industry. Mung bean flour and soybean flour warrian have excellent functional properties and potential to be applied in the food systems (Thompson, 1977; Rosario and Flores, 1981). The ratio of black gram to mung bean flour may vary but blending at the level of 20% has been found to give the best results without affecting much the basic properties of the product (Siddaraju *et al.*, 2008). Mung bean flour blended warrian are very much relished and have great market potential in India and abroad. During the last few years, because of the increasing popularity of these fermented food products in India as well as South East Asian countries, considerable interest has been generated for their export potential but shelf-life, spoilage and the offensive odor developed during storage is a big hinderance. The micro-biological, biochemical and physico-chemical studies on these products are necessary to generate data on changes in the shelf, during transport and, finally, the extent of spoilage (Singh *et al.,* 2013). The ingredients of *Warrian* contain proteins which are readily attacked by proteolytic bacteria (Singh and Kaur, 2006). As no systematic microbiological studies have been carried out on the biochemical changes that proteolytic bacteria bring about in the *Warrian* over time, the present investigation has been undertake.

Materials and Methods

Isolation of Proteolytic bacteria

The spoiled samples of *Warrian* were collected from different local markets during various months of the year, and were immediately processed to isolate proteolytic bacteria. The samples were serially diluted and one ml of the dilution was plated onto skim milk agar (Nutrient agar for bacteria + 10% skim milk) and incubated at 37 °C for 18-24 h. A clear zone around the colony was taken to identify the character of proteolytic microorganisms. Black gram flour warrian was taken as control. The proteolytic microorganisms were transferred and maintained on nutrient agar slants. The samples of warrian were further analyzed for their biochemical activity by intentionally inoculating 3.90 cfu/g of proteolytic bacteria.

Analytical methods

Total solids were calculated by suspending the dough or batter in distilled water, centrifuging it at 8500 g, and drying the supernatant at 75 ºC. Reducing sugars were determined by the dinitrosalicylic acid (DNS) method (Miller, 1972). Soluble proteins were estimated by Lowry's method (Lowry *et al.*, 1951) using (Folin-Ciocalteu) reagent. Proteinase activity was determined according to standard method (Makonen and Porath, 1968), while free amino acids were determined by modified colorimeterical method with Ninhydrin at 570 nm (Soni and Sandhu, 1990). The total free amino acids were expressed as percentage equivalent to leucine. Crude proteins were determined by the Micro-Kjeldahl method (AACC, 1990) .

Packaging and storage studies

The samples of *warrian* along with control were packaged in paper, low density polythene (LDPE), tin containers, glass containers, aluminium laminates, and PET (Polyethylene terephthalate) jars. All packets of the study materials in quadruplicate were heat sealed and subjected to storage tests on the shelf at room temperature for 360 days. The stored samples were analyzed at an interval of 30 days.

Statistical Analysis

All determinations were carried out at least twice and the mean and standard deviation for each of the determinations were calculated and reported. All the data were analysed carried out by one way ANNOVA (Glover *et al.*, 2001).

Results and Discussion

Microbiological and Biochemical studies

During storage studies ,the change in moisture content,appearance ,flavour and proteolytic count were observed.The proteolytic count was observed highest in the third quarter (5.0 $\log_{10}$ cfu g^{-1}) of the year as compared to the control (3.90 $\log_{10}$ cfu g^{-1}) and other quarters of the year. The colony counter (log colonyfarming units\g) of bacteria in first, second and fourth quarter of the year were 4.41, 4.88 and 4.73 respectively (Table 1). An extensive microbiological evaluation of the samples of the mung bean *warrian* revealed the occurrence of proteolytic bacteria along with molds and yeasts. The data on the *warrian* spoilage is scanty but seasonal variations have been found to affect the prevalence of yeasts and bacteria. Large number of mesophilic and thermophilic bacteria grow in fermented products including warrian (Roy et al., 2007, 2011). Bacteria usually dominate in summer while yeasts grow better in winter season (Sandhu *et al.*, 1985 and Soni and Sandhu, 1990). Higher numbers of proteolytic bacteria have been encountered in summer and most of the proteolytic and biochemical activity was observed in the third quarter of the year (Table. 1) which brought significantly higher biochemical changes as compared to the control samples of black gram flour warrian. As compared to the control samples, the pH (4.2), reducing sugar (8.5 mg g^{-1}) and total proteins (22.4 %) were reduced highly in the third quarter while values for total solids (18.5%), soluble proteins (35.5 mg g^{-1}), free amino acids (45.2 ìg g^{-1}) and proteinase activity (7.4 IU g^{-1}) were increased. The values of the parameters were lower in the second quarter as compared to the fourth quarters whereas the values for tota solids, soluble proteins, free amino acids and proteinase activity were observed higher (Table1). In the third quarter of the year the hot and humid weather conditions when temperature ranges between 36-40°C and humidity 80-100% were quite suitable for their growth and protein constituents provided suitable substrates for proteolysis as evidenced by higher values of proteinase activity, decrease in the total protein content and increase in the total free amino acids took place. Moreover, certain proteolytic bacteria produce proteolytic enzymes in their later stages of the life cycle causing spoilage in the products (Boing, 1982). This may be another reason for more proteolytic activity in the third quarter as observed by Singh *et al.*, (2013) in black gram flour warrian. An increase in the free amino acids and soluble proteins is indicative of breakage of the protein network causing brittleness of the products. Certain proteolytic bacteria degrade protein anaerobically producing offensive odor (Dube *et al.*, 2000). The protein degradation products along with ingredient materials may have resulted in the production of off-flavor in the products.

Packaging and Storage Studies

Out of the different types of packaging materials employed for over the year study (Table 3) highest proteolytic count was recorded in Paper (4.25 – 4.75 Log_{10} CFU) and the lowest in PET jars (3.90 – 3.95 Log_{10} CFU). The Proteolytic count was observed higher in LDPE as compared to tin container and glass jar . Al laminated packages had insignificantly higher proteolytic count than PET jar packages . The percent moisture pick up results of mung bean blended warrian depicted in Table 2 show that after 60 days of storage, *warrian* packaged in paper picked up moisture from 0 to 9.3% and there was a gradual rise in moisture uptake after 60 days till 210 days of storage period. Moisture uptake after 210 days of storage period was 13.6%. Warrian packaged in LDPE had low moisture uptake value (5.1%) as compared to paper packaged *warrian* Fig 3. The tin container showed higher moisture uptake as compared to glass jar package, but lower than LDPE. The moisture uptake in tin container after 60 days of storage

Table 1: Microbiological And Biochemical Changes In Mung Bean Flour Blended Warrian During Four Quarters Of The Year

Parameter	Mung Bean flour blended Warrian				
	Control	I	II	III	IV
Proteolytic count (Log_{10} cfu g^{-1})	3.90±0.1^{e}	4.41±0.1^{d}	4.88±0.1^{b}	5.00±0.1^{a}	4.73±0.1^{c}
pH	5.8±0.1^{c}	5.7±0.2^{c}	4.9±0.2^{b}	4.2±0.2^{a}	5.3±0.1^{bc}
Total solids (%)	15.3±0.1^{c}	15.6±0.2^{c}	16.5±0.3^{b}	18.5±0.4^{a}	15.9±0.2^{bc}
Proteinase (IU g^{-1})	6.1±0.2^{c}	6.3±0.2^{b}	6.7±0.2^{b}	7.4±0.3^{a}	6.5±0.2^{b}
Reducing sugars (mg g^{-1})	13.8±0.2^{d}	13.1±0.3^{c}	11.7±0.2^{b}	8.5±0.3^{a}	12.7±0.3^{c}
Soluble proteins (mg g^{-1})	28.6±0.2^{d}	32.5±0.3^{c}	33.4±0.4^{b}	35.3±0.2^{a}	29.3±0.2^{cd}
Total proteins (%)	28.6±0.2^{d}	25.9±0.3^{c}	24.3±0.2^{b}	22.4±0.4^{a}	25.3±0.2^{cd}
Free amino acids (µg g^{-1})	18.3±0.2^{e}	20.4±0.3^{d}	29.3±0.5^{b}	45.2±2.1^{a}	21.8±0.3^{c}
Mean temperature (ºC)	37±2^{b}	17±2^{d}	32±2^{c}	38±2^{a}	10±2^{e}
Mean relative humidity (%)	70±2^{b}	43±2^{e}	57±2^{c}	87±2^{a}	50±2^{d}

Control and experimental samples were prepared in the laboratory by inoculating 3.90 log_{10} cfu g^{-1}, and then incubating the samples for a year. All values have been expressed on dry weight basis and represent averages of 12 samples analyzed in each month. I, II, III and IV represent four quarters of the year i.e. I = January to March; II = April to June; III = July to September; IV = October to December.
The means with similar superscripts in a row do not differ significantly (p> 0.05)

was 0.91% as compared to 0.14% in glass jar . The warrian packaged in PET Jar and Al laminated package showed minor differences in the moisture pick values. The moisture pick up in Al laminated package was noticed marginally higher (0.094% in 210 days) as compared to PET jar (0.089%) after the same storage period . The WVTR values were observed highest for paper package (7.5 $g/m^2/24h$) and lowest for Al laminated package (0.6 $g/m^2/24h$). The sorption studies showed 15.8% EMC in mung bean *warrian* at 67% RH.

Temperature and humidity parameters are of crucial importance for food quality preservation, especially in real life situations, like food market, and house long-life use. Studying the permeability characterization of the different packaging materials for warrian in different environmental conditions is crucial to understand if the selected material is suitable for the long storage of the product or not (Siracusa, 2012). Different packaging materials selected for storing warrian were paper, LDPE, glass jar, tin container, aluminium laminate and PET jar. The mung bean blended warrian packages showed comparatively higher moisture ingress as compared to control samples of black gram flour warrian packages (Table 2). Results correspond to the findings of Pruthi *et al.*, (1983). In a very old publication, Woodward *et al.*, (1960) reported that the introduction of water in a semi crystalline polymer affects principally the amorphous region, breaking-up the hydrogen bonds between the polymer chains and giving, as a consequence, a reduction in the barrier properties of the material. The mung bean warrian have protein content more than that of black gram warrian creating an amorphous condition in the package causing more flow of water in the package than that of black gram package. Obviously higher proteolytic activity that has been observed in mung bean blended warrian as compared to black gram flour warrian may be due to the higher moisture content and physico-chemical composition of the mung bean flour blended warrian. The proteolytic activity was noticed higher in paper package and lowest in Al laminated package because moisture ingress was highest in paper package and least in Al laminated package (Table 3). This makes Al laminates the package of choice for long storage of mung bean warrian. The results correspond to the results obtained by Pruthi *et al.*, (1983) where they found LDPE 300 and 400 gauges quite suitable for packaging black gram and mung bean blended warrian at room temperature, but water vapor transmission rate in LDPE are higher as compared to PET and Al laminates indicating there are more chances of development of proteolytic bacteria in LDPE as compared to Al laminates or PET containers (Suominen *et al.*, 1997) as observed from the proteolytic counts of these materials. From the results obtained in soybean blended warrian it is clear that the packaging material which has low WVTR values ingresses less moisture and thereby low proteolytic activity and *vice versa.* The study reveals PET jar and Al laminates as the best packaging materials for the packaging mung bean flour blended warrian.

Table 2: Mung Bean Flour Blended Warrian Moisture Pick Up (%)

	Days of storage	Control	Paper	LDPE	Glass Jar	Tin Container	PET Jar	Aluminium Laminate
I	30	10.6±0.1[a]	8.2±0.1[a]	2.7±0.2[a]	0.09±0.02[a]	0.58±0.02[a]	0.035±0.002[a]	0.037±0.002[a]
	60	11.3±0.2[b]	9.3±0.2[b]	3.1±0.2[a]	0.14±0.02[b]	0.91±0.02[b]	0.038±0.001[a]	0.042±0.002[b]
	90	12.9±0.2[c]	10.5±0.3[c]	3.4±0.3[b]	0.21±0.02[c]	1.12±0.03[b]	0.045±0.002[b]	0.048±0.002[c]
II	120	16.3±0.2[f]	11.3±0.2[d]	3.8±0.3[c]	0.23±0.02[cd]	1.24±0.03[c]	0.054±0.003[c]	0.058±0.001[d]
	150	17.4±0.2[g]	11.6±0.3[d]	4.1±0.3[c]	0.26±0.02[d]	1.42±0.03[d]	0.066±0.002[d]	0.071±0.002[e]
	180	18.3±0.2[h]	12.8±0.3[f]	4.7±0.3[d]	0.28±0.02[e]	1.61±0.03[f]	0.077±0.002[e]	0.081±0.001[f]
III	210	19.4±0.2[i]	13.6±0.3[h]	5.1±0.3[e]	0.31±0.02[f]	1.72±0.03[g]	0.089±0.002[f]	0.094±0.002[g]
	240	18.4±0.2[h]	12.9±0.3[g]	4.9±0.2[e]	0.27±0.02[de]	1.67±0.03[f]	0.079±0.002[e]	0.086±0.002[f]
	270	18.3±0.2[h]	12.8±0.3[f]	4.7±0.3[d]	0.21±0.02[c]	1.63±0.04[f]	0.075±0.002[e]	0.082±0.002[f]
IV	300	15.1±0.2[e]	12.1±0.3[e]	4.2±0.1[cd]	0.17±0.02[c]	1.53±0.03[e]	0.066±0.002[d]	0.071±0.001[e]
	330	14.2±0.2[d]	11.2±0.2[d]	3.8±0.1[c]	0.13±0.02[ab]	1.43±0.04[d]	0.051±0.002[c]	0.055±0.002[d]
	360	11.1±0.2[b]	10.1±0.3[c]	3.3±0.1[b]	0.11±0.01[a]	1.18±0.03[c]	0.043±0.002[b]	0.048±0.002[c]

Control and experimental samples were prepared in the laboratory by inoculating 3.90 log_{10} cfu g^{-1}, and then incubating the samples for a year. All values have been expressed on dry weight basis and represent averages of 12 samples analyzed in each month. I, II, III and IV represent four quarters of the year i.e. I = January to March; II = April to June; III = July to September; IV = October to December.
* means with similar superscripts in a column do not differ significantly (p> 0.05)

Table 3: Mung Bean Flour Blended Warrian Proteolytic Count*

	Proteolytic Count (Log_{10} (CFU g^{-1})						
	Days of storage	Paper	LDPE	Tin Container	Glass Jar	Aluminium Laminate	PET Jar
I	30	4.26±0.01[a]	4.14±0.02[a]	3.98±0.03[a]	3.97±0.02[a]	3.93±0.01[a]	3.92±0.01[a]
	60	4.42±0.02[b]	4.26±0.02[b]	4.02±0.01[ab]	4.00±0.02[ab]	3.93±0.02[a]	3.92±0.01[a]
	90	4.57±0.03[d]	4.40±0.01[d]	4.06±0.02[b]	4.02±0.02[ab]	3.93±0.01[a]	3.92±0.01[a]
II	120	4.60±0.01[d]	4.46±0.01[e]	4.11±0.01[c]	4.04±0.01[b]	3.94±0.02[a]	3.92±0.01[a]
	150	4.64±0.01[e]	4.55±0.01[f]	4.15±0.01[c]	4.10±0.02[c]	3.94±0.01[a]	3.93±0.01[a]
	180	4.69±0.03[f]	4.57±0.02[f]	4.18±0.03[d]	4.13±0.01[c]	3.95±0.01[b]	3.93±0.02[a]
III	210	4.72±0.02[f]	4.60±0.03[f]	4.18±0.02[d]	4.14±0.02[c]	3.95±0.01[b]	3.93±0.01[a]
	240	4.72±0.03[f]	4.60±0.02[f]	4.13±0.01[c]	4.11±0.01[c]	3.95±0.01[b]	3.93±0.02[a]
	270	4.69±0.02[f]	4.56±0.02[f]	4.09±0.02[b]	4.08±0.03[c]	3.94±0.02[a]	3.93±0.02[a]
IV	300	4.64±0.01[e]	4.46±0.02[e]	4.07±0.03[b]	4.05±0.02[b]	3.94±0.02[a]	3.92±0.01[a]
	330	4.60±0.01[d]	4.42±0.01[d]	4.02±0.01[ab]	4.00±0.01[ab]	3.93±0.01[a]	3.92±0.01[a]
	360	4.50±0.01[c]	4.35±0.01[c]	4.02±0.01[ab]	3.99±0.01[a]	3.92±0.01[a]	3.91±0.01[a]

Control and experimental samples were prepared in the laboratory by inoculating 3.90 log_{10} cfu g^{-1}, and then incubating the samples for a year. All values have been expressed on dry weight basis and represent averages of 12 samples analyzed in each month. I, II, III and IV represent four quarters of the year i.e. I = January to March; II = April to June; III = July to September; IV = October to December.
* means with similar superscripts in a column do not differ significantly (p> 0.05)

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