Physicochemical Properties of Fermented Wheat-Chickpea-Rice Weaning Blend

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Abstract

Purpose- This paper focuses on the effect of fermentation on wheat-chickpea-rice weaning blend.

Design/methodology/approach – Wheat, chickpea, and rice were blended and fermented with *Rhizopus koji* for 60 h at 29±2^{o}C at an interval of 15 h. Blends were analyzed using methods reported in the literature for physicochemical and sensory properties.

Findings – Protein content of the weaning blends increased progressively from 14.63% in the unfermented to 20.89% after 60 hours of fermentation, while the fat content decreased as fermentation time increases. The pH of the samples gradually decreased from 4.53 to 3.83 with gradual increase in titratable acidity as fermentation time increases. The water absorption capacity, total plate count, and yeast and mold count were found to be in the range 112.2±1.16 to 171±1.67%, 1.0×10^{3} to 1.3×10^{3} cfu/g, and 700 to 800 cfu/g, respectively. In terms of sensory analysis, 15 h fermented sample was found to be the best.

Originality/value – The paper has demonstrated effect of fermentation on wheat-chickpea-rice weaning blend.

Keywords: Weaning, *Rhizopus Koji*, wheat, chickpea, rice, Fermentation, Malt, Amylase activity

Article Classification: Research Paper

Introduction

The weaning period also known as food-accustomed period, is the most critical period in the life of infants and preschool children as malnutrition become fairly prevalent among children at that period (Egounlety, 2002; Onyeka and Dibia, 2002). Most traditional weaning foods in developing countries are made from local staple cereals, legumes, starchy fruits, roots, and tubers. Such foods are often of poor protein quality and have high paste properties (Fasasi *et al.*, 2007; Mbata *et al.,* 2006). Moreover, majority of traditional cereal based foods consumed in Africa is processed by fermentation. This has been reported to improve the amino acid composition and vitamin content, increase protein and starch digestibilities, increases the bioavailability of minerals, lower anti-nutrients level, reduced the level of contamination and extends the shelf-life of the food product (Wambugu *et al.*, 2006, Simango, 1997).

Although, commercial baby foods are no doubt nutritionally sound and enriched with micronutrients but in most cases they are prohibitively expensive and are generally not within the reach of poor family. The use of locally available food materials can be used to formulate weaning foods however; studies have shown that these foods are rather bulky. A very convenient alternative to overcome this problem is to produce fermented weaning food which will not only be more digestible and nutritious but also more palatable. This study therefore focuses on the effect of fermentation on wheat-chickpea-rice weaning blend.

Methodology

Materials

The materials used for the research include: Wheat (*Triticum* spp.), rice (*Oryza sativa*), and chickpea (*Cicer arietinum*). These materials were purchased from local market of Dharan, Nepal

Processing of the flours and sample formulation

Whole wheat and chickpea were germinated at room temperature (28 ± 2^{o}C) for 24 hours according to the method of Wang and Field, (1978) followed by drying at 60°C using Ambassador Laboratory Electric Oven (UK) until moisture was reduced to about 6%. Rice on the other hand was roasted at 105°C using the same oven as above till 5-6% moisture content was reached. All samples were milled using Kenwood mixer (Model BL350, PK100/AD England) and sieved to obtain a flour fraction of less than 250µm. Malted wheat flour and malted chickpea flour was added at equal proportion of 2/5 and roasted rice flour was added on the proportion of 1/5 on the final mixture (w/w) and mixed thoroughly. The sample was kept in airtight low density polyethylene (LDPE) pack until use.

*K*oji *preparation*

Pure *Rhizopus* cultures grown at Central Department of Food Technology, Tribhuvan University, Nepal were inoculated in sterilized wheat bran (121^{o}C/15 min) and incubated at 37°C for 48 h at 37°C. The bran with colonies of *Rhizopus* was dried at 45°C for 14 h. Dried *koji* having spores 1.8 x 10^{8} cfu/g was packed in LDPE and stored under refrigerated condition.

Product fermentation and preparation

Mold koji (1%) was added to the calculated amounts of ingredients and product was fermented for 0, 15, 30, 45 and 60 h at 28 ± 2°C in trays by wrapping with sterilized muslin cloth; pH of mash was adjusted to 4.5 with 10% (v/v) lactic acid solution. Fermented product was dried at 60°C for 14 h. Then pan roasting was done at 105°C for 15 minutes until the product was slightly brown. Product was milled in laboratory grinder and sieved with laboratory sieve of 300 microns mesh size. Finally, table Sugar (10%) as a sweetening agent was added.

Chemical analysis

Proximate composition, pH, titratable acidity (TTA), and calcium, were determined using the standard methods described in AOAC (2005). Iron content was determined by colorimetric method (Ranganna, 2001). While, reducing sugar as dextrose, total sugars, total starch and amylase activity were determined by the method of Sadasivam and Manickam (2008).

Physicochemical analysis

Loose and packed bulk densities were determined according to the method of Elkhalifa *et al.* (2005). Viscosity of the product was determined as per AOAC, 2005 using Brookfield DV II viscometer. Water absorption capacity was evaluated by the method described by Afoakwa *et al.*, 2004.

Micobiological analysis

Total plate count was done using nutrient agar media and distilled water as the diluent by pour plate method according to Aneja, 2007. *E. coli* was enumerated in MacKonkey broth as per AOAC (2005).Yeasts and molds count was done in potato dextrose agar as per Aneja (2007).

Sensory evaluation

Sensory evaluation was performed by 9 point hedonic scoring test by 6 female and 10 male semi-trained panelists comprising of graduate students and teachers of Central Campus of Technology, Dharan, Nepal.

Data analysis

Data were represented using Microsoft Excel-2002 and statistically processed by GenStat Discovery Edition 3.

Results and discussion

Effect of fermentation on chemical composition

The chemical composition of different samples having variation in fermentation time is presented in Table 1 below.

Table 1 Effect of fermentation on various chemical composition of weaning food

Parameters	Unfermented	15h	30h	45h	60h
Moisture, %	5.21±0.64[a]	5.21±0.24[a]	5.11±0.07[a]	5.26±0.03[a]	5.35±0.11[a]
Protein, %	14.63±0.3[a]	15.6±0.04[b]	17.79±0.5[c]	18.1±0.15[c]	20.89±0.31[d]
Fat, %	3.55±0.13[a]	2.27±0.06[b]	1.6±0.22[c]	1.36±0.05[d]	1.34±0.17[d]
Ash, %	1.43±0.02[a]	1.54±0.04[a]	1.74±0.06[b]	1.87±0.03[c]	1.77±0.09[bc]
Crude Fiber, %	0.86±0.03[a]	0.91±0.01[ab]	0.87±0.05[a]	0.94±0.02[b]	1.09±0.04[c]
Total Carbohydrate,%	79.53±0.28[a]	79.68±0.04[a]	78±0.65[b]	77.73±0.05[b]	74.91±0.46[c]
Iron, mg	13.43±0.45[a]	13.33±0.31[a]	13.16±0.28[a]	13.31±0.27[a]	13.16±0.18[a]
Calcium, mg	231.67±2.89[a]	232±6.93[a]	230.33±4.04[a]	235±8.89[a]	233±14.73[a]
Reducing Sugar, %	0.3±0.04[a]	1.43±0.05[b]	2.28±0.0551[c]	2.41±0.055[c]	2.41±0.14[c]
Total Sugar, %	7.37±0.92[a]	8.82±0.04[a]	15.94±1.1[b]	25.13±1.78[c]	30.49±0.96[d]
Starch, %	69.21±0.27[a]	67.44±0.49[a]	60.79±1.26[b]	51.77±1.14[c]	43.91±1.13[d]
Amylase activity,%	2.62±0.31[a]	4.45±0.63[b]	4.76±0.56[b]	6.25±0.30[c]	8.7±0.49[d]

[Note: Values are averages of triplicate determination ± s. d.; Values followed by same superscripts within same row are not significantly different ($p>0.05$)**]**.

As shown from the table, crude protein content of the sample increased progressively and significantly from 14.63% in the unfermented to 20.89% after 60 hours of fermentation. An increase in protein content of the fermented blends could be attributed to the breakdown of nutrients of substrates by the *Rhizopus* and there might have been protein synthesis during fermentation thus contributing to the higher values in fermented samples. Similar findings were previously observed by other reporters (Osundahunsi, 2006; Ikujenlola and Fashakin, 2005; Ade-Omowaye *et al*., 2003, Sanni *et al.*1999). On the contrary, the fat content decreased with fermentation time. The decrease in lipid content at the end of fermentation may have resulted from oxidation during fermentation and also could be attributed to the use of lipid by the microorganisms to obtain energy for their metabolic activities during fermentation (Fasasi *et al.*, 2007; Mbata *et al.*, 2006; Sanni *et al.*, 1999; Mensah *et al.*, 1991).

There was also a decrease in the total carbohydrate from 79.53% in unfermented samples to 74.91% after 60 hours of fermentation. Sugar analysis revealed that reducing sugar and total sugar had increasing pattern with fermentation time. There was relatively higher decrease in total starch in fermentation process. This suggested that the amount of starch hydrolyzed to form sugars and organic acids during fermentation is higher. The results of mineral analysis showed no significant difference ($p>0.05$). Although, no significant difference were observed in terms of iron and calcium content between the unfermented and fermented samples, however the calcium content which is required for bone formation was observed to increase by 0.01% after fermentation for 45 hours.

The effect of fermentation on pH and titratable acidity for the samples is as shown in Fig. 1 below. As the fermentation progressed, the pH of samples gradually decreased from 4.53 to 3.83. Conversely, the titratable acidity (TTA) of the product slowly increased with the fermentation time. Probable reason for this may be the formation of carboxylic acid due to proliferation of *Rhizopus* mold (Kunene *et al.*, 1999). This production of carboxylic acid and the consequent rise in acidity is important to avoid proliferation of undesirable organisms during fermentation. Similar decrease in pH and TTA level had been reported by other researchers for different fermented products (Mbata *et al.*, 2006; Olorunfemi *et al.*,

2006; Tetteh *et al.* 2004; Oyewole and Ogundele, 2001; Kunene *et al.*, 1999; Akpapunam and Sefa-dedeh, 1995; Nout *et al.,* 1989).

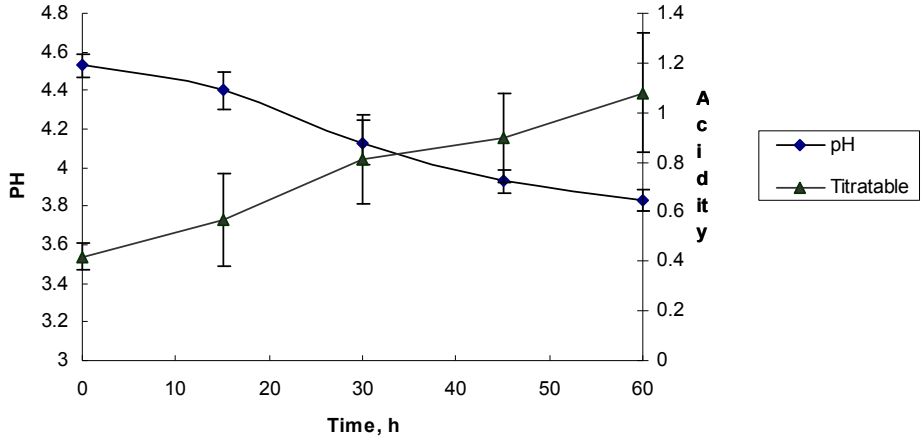

Figure I: Effect of fermentation time on pH and titratable acidity

Effect of fermentation on physical properties

Table 2 gives the loose bulk density, packed bulk density and water absorption capacity of weaning blend from wheat, chickpea and rice fermented for various time periods

Loose bulk density (LBD) decreased from 0.62 in unfermented sample to 0.51 g/mL after fermentation time was increased to 60 hours. Similar decrease was observed for packed bulk density (PBD) for unfermented (0.87 g/mL) and sample fermented for 60 hours (0.76 g/mL). However, water absorption capacity (WAC) of the samples was observed to increase as fermentation time increases. High PBD is important in view of the packaging advantages, as a greater quantity may be packed within a constant volume (Fasasi *et al.*, 2007; Oluwamukomi *et al.*, 2005). The decrease in bulk density of fermented samples would be an advantage as this is required for the preparation of low bulk weaning foods for infants (Elkhalifa *et al.*, 2005).

Table 2 Physical Properties of different products

Parameters	Unfermented	15h	30h	45h	60h
LBD(g/mL)	0.62 ± 0.01^{a}	0.59 ± 0.01^{b}	0.61 ± 0.006^{ab}	0.6 ± 0.012^{ab}	0.51 ± 0.006^{c}
PBD(g/mL)	0.87 ± 0.04^{a}	0.94 ± 0.029^{b}	0.87 ± 0.012^{a}	0.76 ± 0.028^{c}	0.76 ± 0.009^{c}
WAC(%)	112.2 ± 1.16^{a}	132.7 ± 1.33^{b}	132.7 ± 8^{b}	171 ± 1.67^{c}	119.7 ± 3.65^{a}

[Note: Values are average of triplicate determination ± s. d.; Values followed by same superscripts within same row are not significantly different ($p>0.05$)**]**

Viscosities of samples are presented in Fig II. Viscosity analyses showed that viscosity was reduced after 15 h fermentation. An increase only after 30 h fermentation was noted again which was further decreased after 45 h fermentation. Such change might be due to incorporation of malt and fermentation effect as well. An additional hypothesis is that

fermentation breaks down carbohydrates into simpler sugars which do not show the matrix configuration for amylase activity. Similar results were obtained by various workers using malted and fermented cereals. For instance, reductions in viscosities on fermentation of sorghum samples were reported by Taiwo (2009). Furthermore, Wambugu *et al.*, (2006) reported that production of porridges from malted and fermented flours mixes resulted in lower viscosities.

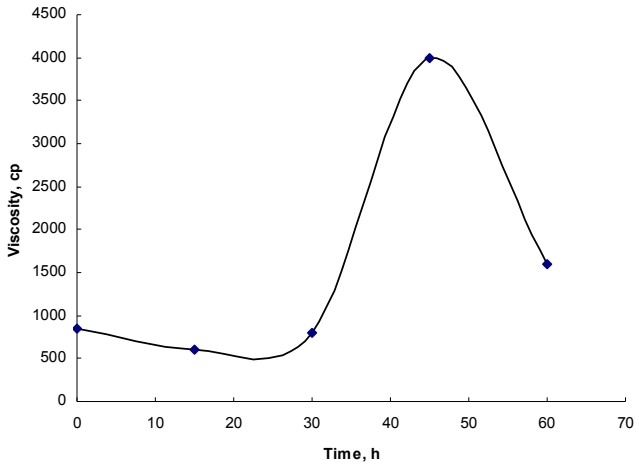

Figure II: Effect of fermentation time on viscosity

Changes in microbial profile

Total plate count, *E. coli*. Yeasts and mold count were determined in prepared samples. Data obtained are presented in Fig 3.

TPC was found to be 1.1×10^3, 1.2×10^3, 1.0×10^3, 1.2×10^3 and 1.3×10^3 cfu/g in samples fermented for 0, 15, 30, 45 and 60 h respectively. The analyses showed that all samples were found to be free of *E. coli* which means the samples are fit for consumption. Samples fermented in intervals of 15 h up to 60 h contain yeast and mold count as 700, 800, 730, 770 and 800 cfu/g respectively. Previous studies had found varying range of microbes in fermented weaning foods. The recipes obtained from the mixtures of the fermented food substrates investigated contained both bacteria and fungi. Olorunfemi *et al.*, (2006) reported that bacterial count of $3.0x10^6$ cfu/g for blends with maize base and $24x10^6$ cfu/g for blends with sorghum base.

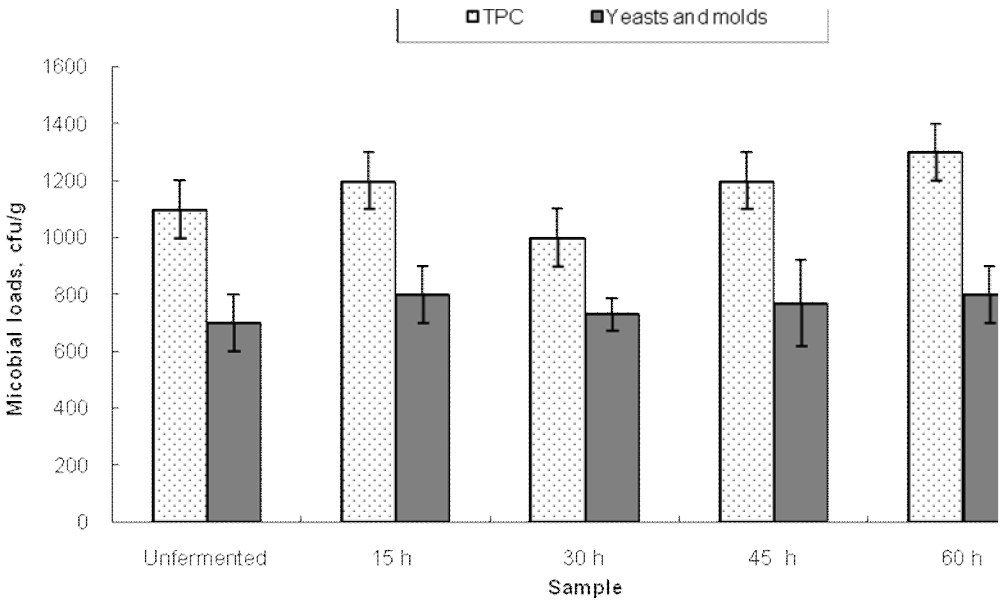

Figure III: Microbial loads on different samples

[**Note:** Values are average of triplicate determination ± s. d.].

Effect of fermentation on sensory quality

Fifteen hour fermentation had no effect on appearance whereas fermentation over 30 h had significant ($p<0.05$) difference. Statistical analyses showed that there was a significant difference ($p<0.05$) in flavor in the samples. Significant difference ($p<0.05$) was noted among fermented samples with different time. Decreasing trend of score after 15 h fermented product revealed that the products had unacceptable flavor on products of 30, 45 and 60 h fermentation. No significant difference between unfermented and 15 h fermented sample as well as between 45 h fermented and 60 h fermented sample for taste. But there was significantly difference between15, 30 and 45 h fermented sample. Decreasing trend of score after 15 h fermented product revealed that the products had unacceptable taste after 30 h fermentation. Statistically there was a significant difference ($p<0.05$) in overall acceptance within the samples. LSD analysis showed that unfermented and 15 h fermented sample had no significant difference ($p>0.05$). But there was significant difference between unfermented and 30 h fermented products. Statistically there was a significant difference ($p<0.05$) in texture within the samples. LSD showed that there was no significant difference ($p>0.05$) between unfermented and fermented samples. But 15 h fermented sample was significant different ($p<0.05$) from 45 h and 60 h fermented samples.

Decreasing trend of score in appearance after 15 h fermented product revealed that the products had unappealing color after 15 h fermentation. This might be due to formation of spores by molds which show black color on the samples. Decreasing pattern was observed for appearance, taste, flavor and overall acceptability after 30 h fermentation. This may be attributed to mold action on the substrate which produces different compounds such as lactic acid, acetic acid, etc. Mensah *et al.*, (1991) reported that fermented weaning foods are more palatable than the bland unfermented products. The consumer acceptability of porridges produced from different fermented blends was rated above average (Sanni *et al.*,

1999). This work demonstrated that sample fermented for 15 hours was the most acceptable in terms of flavor and overall acceptability.

Table 3 Effect of fermentation on sensory attributes

Formulation	Appearance	Flavor	Texture	Taste	Overall
Unfermented	7.12±0.89[a]	7.25±0.68[a]	6.312±0.70[ab]	7.438±0.89[a]	7.125±0.62[a]
15 h	7.25±1.13[a]	7.188±0.83[a]	6.5±0.63[b]	6.875±1.09[a]	7.5±0.82[a]
30 h	6.25±1.0[b]	6.25±0.93[b]	6.25±0.77[ab]	6.065±1.06[b]	6.062±0.57[b]
45 h	5.88±1.2[b]	5.688±1.01[c]	6±0.63[a]	5.438±1.03[c]	5.812±0.54b[c]
60 h	5.62±1.148[b]	5.5±0.97[c]	6.062±0.25[a]	5.75±0.93b[c]	5.562±0.51[c]

[Note: Values are average of triplicate determination ± s. d.; Values followed by same superscripts within same column are not significantly different (p>0.05)**]**

Conclusions

Using *Rhizopus koji* as starter culture, wheat, chickpea, and rice blends could be fermented to prepare weaning food. Improvement of protein content, calcium and water absorption capacity of fermented samples over that of unfermented sample was established in this study. All the samples were observed to be free *from E. coli* which suggested that the samples are fit for consumption. In conclusion, sample that was fermented for 15 hours was found to be more acceptable by the panelist over the other samples. Introduction of this new fermented food formulation as a weaning food to developing Countries may serve as a low cost source of protein to infants.

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