IMPROVEMENT ON TRADITIONAL MILLET FERMENTATION PROCESS AND ITS BREWING QUALITY ASSESSMENT

Dissertation Submitted to the Central Department of Food Technology, Institute of Science and Technology, Tribhuvan University, Nepal, for the Award of Doctor of Philosophy (Ph.D.) Degree in Food Technology

By

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Recommendation

This is to certify that **Mr. Dhan Bahadur Karki** has completed dissertation entitled **Improvement on Traditional Millet Fermentation Process and Its Brewing Quality Assessment** for the award of the degree of Doctor of Philosophy (Ph.D.) in Food Technology under my supervision. To my knowledge, this research work has not been submitted for any other degree elsewhere.

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Date: 20 May 2013

Declaration

I hereby declare that the work presented in this dissertation has been done by myself and has not been submitted for the award of any degree elsewhere. All the sources of information used in this work have been acknowledged and fully cited in the bibliography section.

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Dhan Bahadur Karki

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Abstract

Defined alcoholic fermentation starter for cereal fermentation was prepared using *R. oryzae* (ITCC N0. 4408) mold and *S. cerevisiae* yeast in wheat bran-rice flour mixture (25:75) and used for cereal fermentations. Effects of fermentation containers and raw materials on the quality of fermented cereals were studied. Finger millet (var. *Kabre*) was dehusked, cleaned, washed, soaked for 2 h, cooked, and cooled to room temperature. Fermentation starter was added at the rate of 1% by weight of millet; biomass developed for 2 days at 29 ± 1 ºC, filled into different containers and fermented at 26 ± 1 ºC for 15 days. Chemical and sensory quality of fermented millet, rice, maize, and wheat were also compared. Rice and wheat were incorporated to millet (singly and in combination) and their effects on the quality of fermented millet were studied.

Quality comparison between solid - and semi solid-state fermentations (biomass developed millet : water :: 1:0.5 and 1:1 m/v respectively) was carried out. Biochemical changes during millet fermentation, storage stability of fermented millet packed in polyvinylchloride (PVC) container at room temperature (25 ± 2 ºC), and clarification of millet *jand* using fining agents were studied. Chemical and sensory quality of fermented millet using defined starter were compared with those found in the market.

Alcohol (15 ± 0.8% v/m) and total ester (0.85 ± 0.06 g ethyl acetate /L alc) contents between plastic and wooden containers were not different ($p>0.05$), while earthen container resulted significantly lower alcohol (11.21% v/m) and higher total esters (1.831 g ethyl acetate /L alc) in the fermented millet. Millet fermented in earthen container had total aldehydes content by more than three times (0.850 g acetaldehyde /L alc) than that of plastic container. Total esters, TSS, total and fixed acidities and pH were significantly higher in wheat *jand*, while no remarkable difference in alcohol content was found among millet, rice, maize, and wheat *jands*. Sensory quality of rice *jand* was liked very much, whereas that of maize was disliked slightly. Total ester decreased by wheat addition, total aldehydes increased by both rice and/or wheat addition, while methanol content remained unaffected by cereal combination. Addition of wheat significantly impaired ($p<0.05$) the taste and smell of millet *jand*.

Results of solid versus semi-solid fermentations revealed that total esters, total-, free- and volatile acidities increased remarkably, while alcohol remained unaffected by semi-solid fermentation of finger millet compared to solid-state one. Sensory evaluation

revealed that taste, color, and smell of fermented millet were significantly impaired by semi-solid state fermentation. Chemical analysis of fermented finger millet packed in PVC container and kept at 25 ± 2 ºC for 90 days showed that total aldehydes, esters and reducing sugar increased ($p< 0.05$) by 24, 58 and 13% respectively, whereas alcohol content decreased by 9% over the storage period. Remarkable improvement in sensory quality of fermented millet occurred during room temperature storage.

Different fining agents (bentonite, gelatin, tannin and tannin-gelatin combination) were tried for the clarification of finger millet *jand*. Addition of bentonite (3 g/L) resulted the best clarification of all the fining agents without significantly affecting the chemical and sensory quality of the clarified *jand*, while tannin-gelatin combination showed an adverse effect on *jand* clarification. The results of biochemical changes during millet fermentation showed that moisture, TSS, acidity and sugars increased, while starch decreased during fermentation. About 69% of the total alcohol was formed on day 6 with a final alcohol content of 14.58% (v/m) on day 12. Finger millet fermented for 3 and 6 days had total aldehydes (as acetaldehyde), esters (as ethyl acetate), methanol and fusel oil contents of 1.857 and 1.089, 2.121 and 1.124, 1.753 and 1.5 and 8.028 and 4.366 g/L alc respectively. Total oxalate decreased by 51% on day 6, phytic acid decreased by 4.5-fold on day 12, while total free amino acids increased by 6-fold on day 6 during fermentation. Phosphorous, manganese, sodium, potassium, and zinc contents increased while iron did not changed by fermentation.

Chemical analysis of fermented millet using defined starter (lab sample) and traditional starter (market sample) revealed that lab sample had higher alcohol, but similar fusel oil contents compared to market sample, while market sample had alarmingly higher total aldehydes (0.305 – 0.390 g acetaldehydes/L alc) and methanol (3.723 – 5.840 g/L alc) contents than that of lab sample. Millet fermented with defined starter had superior sensory quality than that fermented by traditional starter.

Brewing potential of six Nepalese finger millet varieties was investigated. Millets were soaked for 12 h at room temperature (26 – 28 ºC), germinated for different times at 28 ±1 ºC, dried at 50 ± 2 ºC for 24 h and enzymatic activities and chemical characteristics of the malts were analyzed. Effect of malt kilning temperature and kilning methods on malt quality; mashing methods, barley malt and mold bran additions on wort properties, and gibberellic acid treatment during seed germination on malt quality were studied. Changes during millet and barley beer fermentation, and quality comparison between

barley and millet beers were carried out. Mashing condition for millet malt was optimized using response surface methodology.

Alpha-amylase activity was maximum in 72 h germinated *Dalle* millet malt (22. 96 units/g dry malt), while beta-amylse activity was maximum in 48 h germinated *Kabre* millet malt (385 units/g dry malt). Forty-hour germinated *Juwain* millet exhibited the highest carboxypeptidase activity (242.5 units/g dry malt) and FAN contents (57.8 mg glycine/100 g dry matter). Amylose to amylopectin ratios in native and malted millet starch were 29:71 and 28:72 respectively. Malt extract analysis showed that color, free amino nitrogen (FAN as glycine), and total reducing sugar (as maltose) ranged between 2.77 – 5.78 EBC units, 2.6 – 9.0 mg% (m/v) and 4.50 – 6.93 mg% (m/v) respectively among the six millet malt extracts. Higher kilning temperature (80 ± 2 ºC) significantly decreased ($p<0.05$) α- amylase and carboxypeptidase activities, while β-amylase activity and FAN did not change compared to 50 ± 2 ºC kilned millet malt. Malt extract color increased, while FAN decreased with increasing kilning temperature.

Incorporation of barley malt up to 40% (m/m) to millet malt did not improve the wort properties appreciably, while FAN increased by 58% over the control. Addition of mold bran to millet malt up to 2.5% reduced wort viscosity by 8% and increased FAN and formol nitrogen by 81 and 44% respectively in the wort compared to control. The US mashing process produced the highest reducing sugar (9.27%, m/v as maltose), FAN (17.4 mg% , m/v as glycine) and dextrin (3.49%, m/v) in the wort compared to infusion mashing at 70 ºC and decantation mashing at 80 ºC.

Evolution of TSS during millet and barley malts mashing using US mashing process revealed that millet starch had gelatinization temperature around 70 ºC. Optimization of mashing temperature and pH for millet malt using response surface methodology in the US mashing process showed that the optimum temperature and pH for protein rest, dextrinizing and conversion periods were 57.62 ºC and 5.47, 68 ºC and 4.5, and 70 ºC and 5.28 respectively.

Gibberellic acid (GA_3) treatment (5-ppm solution in water) during millet germination at 28 ± 1 ºC significantly increased amylase activities and FAN contents in all millet malts compared to control. *Kabre* millet variety exhibited a strong response to GA_3 in increasing enzyme activities and FAN, and produced better malt when germinated for 56 h of all millet varieties studied.

Results of chemical changes during millet and barley beer fermentation showed that most of the chemical properties of the wort substantially changed over the first three days of fermentation in both millet and barley malt worts; however, the changes were relatively faster in barley beer. Fusel oil and methanol decreased, while total aldehydes and esters increased with fermentation time.

Chemical and sensory analyses of millet and barley beer showed that dextrin content was lower in barley beer (0.89% m/v) than in millet beer (average of 1.42% m/v). Total phenolics content (as gallic acid) was 59.2 mg% (m/v) in barley beer, while it was 48.6 mg% (m/v) (average of two beers) in millet beer. Formaldehyde content was higher in barley beer (0.33 ppm) than in millet beer (average of 0.235 ppm). Fusel oil content was lower in barley beer (228.85 g/100 L alc), while menthol (249.71 g/100 L alc) and total aldehydes (32.23 g/100 L alc) were higher in barley beer compared to millet beers. Vicinal diketones content was higher in barley beer (0.44 ppm) than in millet beer (average of 0.3 ppm). Sensory quality of millet beer was comparable to that of barley beer, except that body of millet beer was better compared to barley beer.

Keywords: Millet, defined starter, traditional fermentation, brewing potential, analyses

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