Asian Journal of Bio Science, **Vol. 3 No. 2 : 311-316 (October, 2008)**

Utilization of minor millets for tempeh production

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(Accepted : August, 2008)

An experiment was carried out for the utilization of minor millets (little foxtail and finger millet) with the soybean and horse gram for millet tempeh production. *Rhizopus microsporus* var *oligosporus* was grown satisfactorily on the substrates. At 35^0C fermentation was completed within 39.50 hours compared to 30^0C (53.14 hours). The pH, which was adjusted at about 5.2-5.5 with acetic acid in the substrates and during fermentation, raises progressively upto 48 hrs of incubation period to above 7.1-7.3, which was maintained constantly. The Acid protease activity was found to be increased with increase in time intervals upto 36 hrs beyond which, it decreased significantly. But, Acid protease activity was significantly highest at 35^0C as compared to 30^0C.

Key words : Tempeh, Acid protease, *Rhizopus microsporus* var. *oligosporus*, Fermentation, Soybean, Horsegram, Minor millets.

INTRODUCTION

India attained self-sufficiency in food production to meet the required quantity. However, malnutrition is one of the nutritional constraints mainly due to protein deficiency in the country and in other developing countries like India. In order to solve the problem of malnutrition (protein hunger), possible sources of protein production shall have to be exploited to meet the challenge. Exploitation of traditional food resources can make substantial break through to meet protein deficiency. Small millets as a group include several coarse cereals namely finger millet, little millet, foxtail millet, kodo millet, proso millet and barnyard millet grown throughout the length and breadth of the country in diverse soils and climatic conditions *i.e.*, in wide range of climates and are cheap in cost. The area under small millet in India is around 4.0 million hectares with the production of 3.6 million tones. In Karnataka, annually these crops are grown over an area of 1.3 million hectares with the production of 1.6 million tones. Small millets have remained as the food for the people of lower socioeconomic strata and traditional consumers. Grains are rich in minerals and fiber content. Recent studies indicate that minor millets are nutritionally superior to conventional food grains and exhibit hypoglycemic effect due to presence of higher proportion of unfavorable complex carbohydrate, resistant starch and release sugars slowly (Malleshi, 1993 and Mani *et al.*, 1993). The flavour and difficulty in processing of millets are the limitations for their use in the routine diets. Pulses like soybean and horse gram are not eaten in raw state, but are processed in a number of ways before consumption, which may have an effect on their nutritional quality and digestibility of nutrients (Kalmesh *et al.*, 2002).

MATERIALS AND METHODS

Soybean (*Glycine max*) and horse gram (Dolichos biflorus) were obtained from Main Agricultural Research Station, University of Agricultural Sciences, Dharwad. Minor millets like foxtail millet (*Setaria italica* var HMT-100-1), little millet (*Panicum milearum* var. TNAU-63) and finger millet (*Eleucine coracana* var GPU-34) obtained from the A.R.S, Hanumanamatti. Culture organism (*Rhizopus microsporus* var. *oligosporus* MTCC-556) was obtained from the culture collection center, IMTECH, Chandigarh. Chemicals used for the research were of analytical grade. Soybean and horse gram were dehulled by soaking in the water for over night and rubbing with hand and hulls removed by flotation method. The fungal culture, *Rhizopus microsporus* var. *oligosporus* was maintained on slants of potato dextrose agar at 4^0C. Before each experiment, the fungus was transferred to fresh PDA slants and incubated at 25^0C for 7 days. Millet tempeh was prepared by using soybean and horse gram at different proportions with millets. The treatments are T1 (100% pulses), T2 (75% pulses + 25% millets), T3 (50% pulses + 50% millets), T4 (25% pulses + 75% millets), T5 (100% millets). Before inoculation of fungal spore suspension, the pH of the substrate was adjusted using acetic acid at 2.4 ml per 100 g of substrate to maintain the pH for the convenient growth of fungi. The pH of each treatment was recorded initially and after incubation of treatments at 30^0C, 35^0C and 40^0c at

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different time intervals (12, 24, 36, 48, and 60 hrs). Acid protease activity was measured according to the hemoglobin digestion method described by Anson (1938). Reaction mixture containing 1 ml of 1% dialyzed hemoglobin in 0.05M sodium phosphate buffer at pH 4.0 and 1 ml of properly diluted culture extract was incubated at 50^{0}C for 2 hrs. The reaction was stopped by the addition of 3 ml of 5% trichloro acetic acid. The undigested hemoglobin was removed by the Lowry's method (Lowry *et al.*, 1951).

RESULTS AND DISCUSSION

Growth of *Rhizopus oligosporus* on the substrates incubated at different temperatures was presented in Table 2. The results revealed difference between temperature ranges (30, 35 and 40^{0}C). Among them *Rhizopus* shown growth at 30 and 35^{0}C. But, growth was not observed at 40^{0}C indicating that 30 and 35^{0}C were found to be optimum temperatures for fermentation of millet tempeh (Table 2). The temperature within the fermenting beans mass does not raise above approximately 40^{0}C or the high temperature may damage subsequent growth of the mold (Han *et al.*, 2001). Incubation temperature of 37^{0}C favoured the growth of *R. oligosporus* while being less favorable for growth of the mesophilic molds and also fewer bacterial species grow at 35 to 37^{0}C than at 32^{0}C, but *Klebsiella pneumoniae* rapidly at the higher temperature.

The data recorded on the fermentation of millet tempeh supplemented with soybean and horse gram at different temperatures are presented in Table 1. The data clearly showed that different temperatures had positive influence on fermentation of millet tempeh. However, the fermentation was significantly superior at 35^{0}C (39.5 hrs)

Table 1 : Effect of incubation temperature on pH during fermentation of soybean and horsegram supplemented with millets at different proportions at different time intervals

Substrates	Temperature (^{0}C)	Time interval (hrs)						
		0	12	24	36	48	60	Mean
T_1 : 100% soybean	30	5.40	8.05	6.20	6.50	6.88	7.30	6.18
	35	5.50	6.05	6.30	6.60	7.10	0.00	6.28
	Mean	5.45	6.05	6.25	6.55	6.99	7.30	6.23
T_2 : 75% soybean + 25% foxtail millet	30	5.50	5.70	6.10	6.40	6.80	7.30	6.16
	35	5.50	5.80	6.20	6.50	7.20	0.00	6.26
	Mean	5.55	5.75	6.15	6.45	7.00	7.30	6.21
T_3 : 50% soybean + 50% foxtail millet	30	5.60	5.90	6.20	6.50	6.92	7.40	6.26
	35	5.50	6.00	6.30	6.60	7.32	0.00	6.36
	Mean	5.55	5.95	6.25	6.55	7.12	7.42	6.31
T_4 : 25% soybean + 75% foxtail millet	30	5.60	5.80	6.00	6.40	6.93	7.30	6.16
	35	5.60	5.90	6.10	6.50	7.10	0.00	6.24
	Mean	5.65	5.85	6.05	6.45	7.01	7.30	6.20
T_5 : 100% foxtail millet	30	5.83	5.90	6.00	6.50	6.80	7.43	6.31
	35	5.90	6.00	6.30	6.60	7.20	0.00	6.40
	Mean	5.86	5.95	6.25	6.55	7.04	7.43	6.36
T_6 : 75% soybean + 25% little millet	30	5.50	5.90	6.10	6.50	6.98	7.20	6.19
	35	5.65	6.00	6.20	6.60	7.20	0.00	6.29
	Mean	5.51	5.95	6.15	6.55	7.09	7.20	6.24
T_7 : 50% soybean + 50% little millet	30	5.59	5.80	6.00	6.60	6.96	7.40	6.22
	35	5.60	5.90	6.10	6.70	7.30	0.00	6.32
	Mean	5.59	5.85	6.05	6.65	7.13	7.40	6.27
T_8 : 25% soybean + 75% little millet	30	5.40	5.80	6.20	6.50	6.99	7.33	6.18
	35	5.48	5.90	6.30	6.60	7.10	0.00	6.22
	Mean	5.44	5.85	6.26	6.55	7.04	7.33	6.20
T_9 : 100% little millet	30	5.60	5.90	6.13	6.60	6.99	7.27	6.26
	35	5.70	5.90	6.16	6.70	7.20	0.00	6.36
	Mean	5.65	5.90	6.15	6.65	7.09	7.27	6.31
T_{10} : 75% soybean + 25% finger millet	30	5.60	6.00	6.10	6.40	6.90	7.40	6.18

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T_{10} : 75% soybean + 25% finger millet	30	5.60	6.00	6.10	6.40	6.90	7.40	6.18
	35	5.70	5.90	6.16	6.50	7.06	0.00	6.28
	Mean	5.65	5.95	6.13	6.45	6.98	7.40	6.23
T_{11} : 50% soybean + 50% finger millet	30	5.50	6.00	6.26	6.60	7.06	7.30	6.22
	35	5.60	5.80	6.30	6.50	7.13	0.00	6.32
	Mean	5.55	5.85	6.28	6.55	7.09	7.30	6.27
T_{12} : 25% soybean + 75% finger millet	30	5.30	5.90	6.36	6.36	6.55	7.50	6.16
	35	5.40	5.70	6.36	6.60	6.93	0.00	6.26
	Mean	5.35	5.75	6.36	6.60	7.01	7.50	6.21
T_{13} : 100% finger millet	30	5.70	5.80	6.33	6.70	6.99	7.50	6.40
	35	5.80	5.80	6.36	6.50	7.30	0.00	6.46
	Mean	5.75	5.80	6.35	6.65	7.14	7.50	6.43
T_{14} : 100% horsegram	30	5.00	6.10	6.23	6.50	7.05	7.40	6.14
	35	5.00	6.00	6.33	6.60	6.93	0.00	6.22
	Mean	5.00	6.05	6.28	6.55	6.99	7.40	6.18
$_{T15}$: 75% horsegram + 25% foxtail millet	30	5.16	5.90	6.30	6.60	7.06	7.50	6.18
	35	5.40	6.00	6.33	6.72	7.20	0.00	6.28
	Mean	5.28	5.95	6.31	6.66	7.13	7.50	6.23
T_{16} : 50% horsegram + 50% foxtail millet	30	4.90	5.80	6.20	6.53	6.99	7.50	6.08
	35	5.00	5.90	6.30	6.46	7.10	0.00	6.18
	Mean	4.95	5.85	6.28	6.56	7.04	7.50	6.13
T_{17} : 25% horsegram + 75% foxtail millet	30	5.10	5.90	6.33	6.43	6.97	7.40	6.16
	35	5.20	6.00	6.36	6.43	7.10	0.00	6.26
	Mean	5.15	5.95	6.35	6.43	7.03	7.40	6.21
T_{18} : 75% horsegram + 25% little millet	30	5.10	5.90	6.30	6.60	6.92	7.50	6.20
	35	5.20	6.00	6.33	6.70	7.13	0.00	6.30
	Mean	5.15	5.95	6.31	6.65	7.23	7.50	6.25
T_{19} : 50% horsegram + 50% little millet	30	4.90	6.00	6.23	6.60	7.18	7.40	6.16
	35	5.00	6.10	6.30	6.55	7.22	0.00	6.26
	Mean	4.95	6.05	6.28	6.51	7.60	7.40	6.21
T_{20} : 25% horsegram + 75% little millet	30	5.10	5.80	6.30	6.60	6.90	7.50	6.15
	35	5.20	5.90	6.34	6.55	7.26	0.00	6.24
	Mean	5.15	5.85	6.33	6.50	7.08	7.50	6.19
T_{21} : 75% horsegram + 25% finger millet	30	5.10	6.00	6.24	6.50	6.98	7.50	6.19
	35	5.20	6.10	6.30	6.60	7.11	0.00	6.29
	Mean	5.15	6.05	6.31	6.55	7.04	7.50	6.24
T_{22} : 50% horsegram + 50% finger millet	30	5.10	6.12	6.23	6.60	6.95	7.40	6.18
	35	5.10	6.20	6.26	6.70	7.17	0.00	6.28
	Mean	5.10	6.16	6.25	6.65	7.06	7.40	6.23
T_{23} : 25% horsegram + 75% finger millet	30	5.10	5.90	6.17	6.50	7.60	7.30	6.18
	35	5.10	6.00	6.23	6.60	7.24	0.00	6.26
	Mean	5.10	5.95	6.20	6.55	7.12	7.30	6.22
			S.E.±			C.D. (P=0.01)		
Substrates (A)			0.0196			0.054		
Temperature (B)			0.0058			0.0160		
Time interval (C)			0.058			0.0160		
A × B			0.0439			0.1216		
A × C			0.0278			0.0770		
B × C			0.0130			0.0360		
A × B × C			0.0621			0.1721		

Table 2 : Acid protease activity of *Rhizopus microsporus* var. *oligosporus* on the substrates at different temperatures and time intervals

Substrates	Temperature (^{0}C)	Time interval (hrs)					Mean
		12	24	36	48	60	
T_1 : 100% soybean	30	6.84	78.27	92.56	97.16	9.14	53.23
	35	7.14	94.84	98.13	12.56	0.00	68.70
	Mean	7.12	86.55	95.34	54.86	9.14	60.97
T_2 : 75% soybean + 25% foxtail millet	30	5.91	91.71	81.16	87.31	6.57	50.30
	35	6.73	88.11	86.10	10.21	0.00	58.85
	Mean	6.32	74.91	88.63	48.82	6.57	54.58
T_3 : 50% soybean + 50% foxtail millet	30	2.72	54.84	68.56	74.27	3.29	45.81
	35	4.33	83.11	86.84	7.41	0.00	50.69
	Mean	3.53	69.12	77.70	40.84	3.29	48.25
T_4 : 25% soybean + 75% foxtail millet	30	2.72	52.56	65.13	68.56	4.60	38.74
	35	4.33	64.26	80.11	6.27	0.00	47.24
	Mean	3.53	58.41	72.62	37.41	4.46	42.99
T_5 : 100% foxtail millet	30	1.97	37.71	49.13	67.41	2.59	32.94
	35	2.96	54.84	69.16	4.56	0.00	39.13
	Mean	2.46	46.27	59.34	35.98	2.59	36.04
T_6 : 75% soybean + 25% little millet	30	5.83	72.10	85.71	84.13	5.78	46.36
	35	6.56	81.13	88.33	9.27	0.00	61.94
	Mean	6.19	76.62	81.02	46.70	5.78	54.13
T_7 : 50% soybean + 50% little millet	30	5.11	60.56	78.84	74.27	4.25	41.75
	35	4.53	77.71	78.36	6.42	0.00	54.69
	Mean	4.82	69.13	78.60	40.34	4.25	48.23
T_8 : 25% soybean + 75% little millet	30	2.51	55.17	67.41	72.11	3.20	35.72
	35	4.23	63.27	70.16	5.23	0.00	48.55
	Mean	3.37	57.72	68.78	38.67	3.20	42.14
T_9 : 100% little millet	30	1.51	43.41	60.56	64.84	3.21	30.86
	35	2.52	53.56	62.37	5.01	0.00	42.58
	Mean	2.01	48.49	61.46	34.92	3.21	36.72
T_{10} : 75% soybean + 25% finger millet	30	5.76	56.11	77.70	73.13	4.92	41.43
	35	5.91	72.03	88.13	10.24	0.00	55.82
	Mean	5.83	64.07	82.91	41.68	4.92	48.63
T_{11} : 50% soybean + 50% finger millet	30	3.11	50.27	68.22	65.18	3.83	36.21
	35	5.41	65.13	74.27	8.41	0.00	48.78
	Mean	4.26	57.70	71.24	36.79	3.83	42.50
T_{12} : 25% soybean + 75% finger millet	30	5.20	42.27	49.13	54.84	2.20	30.23
	35	2.97	52.16	59.82	5.97	0.00	37.18
	Mean	2.73	47.21	54.21	30.40	2.20	33.71
T_{13} : 100% finger millet	30	1.91	35.41	41.13	43.41	1.19	24.95
	35	2.11	42.33	51.41	4.11	0.00	30.96
	Mean	2.01	38.87	46.27	23.76	1.19	27.96
T_{14} : 100% horsegram	30	4.99	60.56	70.84	78.22	8.13	43.06
	35	5.63	73.13	84.36	9.13	0.00	53.64
	Mean	2.30	66.84	77.60	43.67	8.13	48.36
T_{15} : 75% horsegram + 25% foxtail millet	30	3.51	46.84	54.84	75.31	7.24	39.51
	35	4.81	67.41	77.71	8.12	0.00	45.12
	Mean	4.16	57.12	66.27	41.71	7.24	42.36
T_{16} : 50% horsegram + 50% foxtail millet	30	2.68	38.64	51.27	71.81	4.35	36.05
	35	3.95	63.41	69.83	7.01	0.00	41.10
	Mean	3.31	51.02	60.55	39.41	4.35	38.58

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T_{17} : 25% horsegram + 75% foxtail millet	30	2.63	33.71	49.91	68.71	3.13	33.72
	35	3.55	59.91	64.21	5.21	0.00	38.73
	Mean	3.09	46.81	57.06	36.96	3.13	35.98
T_{18} : 75% horsegram + 25% little millet	30	3.31	52.56	64.11	64.11	5.23	38.06
	35	4.13	64.56	76.27	8.11	0.00	46.22
	Mean	3.72	58.56	70.19	36.11	5.23	42.15
T_{19} : 50% horsegram + 50% little millet	30	2.42	43.40	49.13	60.86	4.56	34.98
	35	3.61	60.81	69.47	6.91	0.00	39.25
	Mean	3.01	52.11	59.30	33.88	4.56	37.12
T_{20} : 25% horsegram + 75% little millet	30	1.97	35.41	41.13	58.91	3.18	32.11
	35	2.11	56.73	64.46	5.16	0.00	35.18
	Mean	2.04	46.07	52.79	32.03	3.18	33.68
T_{21} : 75% horsegram + 25% finger millet	30	1.97	56.12	64.16	70.37	4.84	34.99
	35	3.74	64.56	68.26	7.41	0.00	48.15
	Mean	2.85	60.34	66.21	38.89	4.84	41.57
T_{22} : 50% horsegram + 50% finger millet	30	1.01	46.27	54.75	62.96	3.62	30.50
	35	2.11	58.61	59.42	6.55	0.00	42.41
	Mean	1.56	52.44	57.08	34.75	3.62	36.46
T_{23} : 25% horsegram + 75% finger millet	30	0.88	42.17	56.01	53.83	2.19	26.59
	35	1.81	51.88	47.81	4.88	0.00	38.22
	Mean	1.34	47.02	51.91	29.35	2.19	32.41

	S.E.±	C.D. (P=0.01)
Substrates (A)	0.0914	0.2533
Temperature (B)	0.0269	0.0745
Time interval (C)	0.0381	0.1055
A × B	0.1827	0.5063
A × C	0.1292	0.3580
B × C	0.0539	0.1493
A × B × C	0.2581	0.7161

indicating that fermentation of millet tempeh was faster as compared to 35^{0}C (53.14 hrs) T_1 (100% soybean) recorded lowest incubation period of 42 hrs. The treatments T_1, T_3, T_8, T_{15}, and T_{17} were not differ significantly at 35^{o}C indicating less incubation period for fermentation of millet tempeh. Han *et al.* (2002) observed that ambient temperatures of 30^{0}C and 35^{0}C during the summer season prohibit the use of the usual starter *Actinomucor elegans* and *Rhizopus oligosporus* were optimum at 25^{0}C at relative humidity of 95.97 % and 35^{0}C at relative humidity 95 to 97 %, respectively (Table 1).

The data showed with respect to incubation temperature on pH during fermentation of soybean and horse gram supplemented with millets are presented in Table 3. The change in pH of the substrate during fermentation at different time intervals increased with increase in time intervals from 0 hr to 48 hrs, beyond which, it remained constant. However, the fermentation was maximum at 35^{0}C at in different time intervals. Further, the fermentation of millet tempeh was found to be in treatment receiving 100 per cent foxtail millet (T_5; 6.36).

The data shown with respect to incubation temperature on acid protease activity during fermentation of soybean and horse gram supplemented with millets at different proportions at different time intervals are presented in Table 3. Acid protease activity was found to be increased with increase in time intervals upto 36 hrs beyond which, it decreased significantly. But, acid protease activity was significantly highest at 35^{0}C as compared to 30^{0}C. further, acid protease activity was maximum and significantly superior in treatments receiving 100% soybean (60.97µg of tyrosine liberated/hour/mg of protein) as compared to treatment combinations. But it was maximum at 36 hrs (72.56µg of tyrosine liberated/hr/mg of protein) when supplemented with 100% soybean alone. Wang and Hesseltine (1965) reported two proteolitic enzyme systems in the culture filtrates of *R. oligosporus*. One has on optimum pH at 3.0 and the other at 5.5. Both the enzymes have maximum activities at 50 and 55^{0}C and

Table 3 : Time taken for complete growth of *Rhizopus nucrosporus* var. *oligosporus* on the substrates incubated at different temperatures

Substrates	Time taken for complete growth (hrs)		
	30^0C	35^0C	Mean
T_1 : 100% soybean	49.00	35.00	42.00
T_2 : 75% soybean + 25% foxtail millet	52.00	37.00	44.50
T_3 : 50% soybean + 50% foxtail millet	51.00	35.00	43.00
T_4 : 25% soybean + 75% foxtail millet	48.00	38.00	43.00
T_5 : 100% foxtail millet	52.00	40.00	46.00
T_6 : 75% soybean + 25% little millet	51.00	38.00	44.50
T_7 : 50% soybean + 50% little millet	49.00	38.00	43.50
T_8 : 25% soybean + 75% little millet	50.0	35.00	42.50
T_9 : 100% little millet	51.00	39.00	45.00
T_{10} : 75% soybean + 25% finger millet	61.00	41.00	51.00
T_{11} : 50% soybean + 50% finger millet	58.00	43.00	50.50
T_{12} : 25% soybean + 75% finger millet	62.33	47.00	54.00
T_{13} : 100% finger millet	61.00	47.00	42.50
T_{14} : 100% horsegram	48.00	37.00	43.50
T_{15} : 75% horsegram + 25% foxtail millet	52.00	35.00	43.16
T_{16} : 50% horsegram + 50% foxtail millet	51.00	35.33	43.00
T_{17} : 25% horsegram + 75% foxtail millet	51.0	35.00	45.00
T_{18} : 75% horsegram + 25% little millet	52.00	38.00	45.00
T_{19} : 50% horsegram + 50% little millet	52.00	37.00	46.00
T_{20} : 25% horsegram + 75% little millet	51.00	41.0	45.00
T_{21} : 75% horsegram + 25% finger millet	52.00	38.00	49.50
T_{22} : 50% horsegram + 50% finger millet	57.00	42.00	54.50
T_{23} : 25% horsegram + 75% finger millet	61.00	48.00	54.50
Mean	53.14	39.50	
	S.E.±		C.D. (P=0.01)
Substrates (A)	0.407		0.120
Temperature (B)	0.120		0.300
Interaction (A x B)	0.575		1.594

are fairly stable at pH 3.0 to 6.0. Wang *et al.* (1974) reported that growth conditions for maximum proteins production by *R. oligosporus*, *Mucor disperses* and *Actinomucor elegans* and enzyme yields by all three fungi were higher in solid substrate fermentations than in submerged cultures.

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