Optimization of growth conditionsfor tempehproduction from sorghum and protease activity.

ABSTRACT

Fermentation improves the nutraceutical properties of cereals. The objective of this work was to optimize the growth condition for tempeh production from sorghum. Tempeh was developed with sorghum and in combination with soybean. Effective proliferative growth of *Rhizopus oligosporus* (*R. oligosporus*)was found on all the substrates. The temperature had a significant effect on the growth of the mould, pH, and protease activity. The incubation temperature of 35^{O}c was found to be more favourable than 30^{o}C. The fermentation at 35^{o}Cwas completed within 36 hours compared to 30^{o}C (46 hrs). The pH, was raised progressively up to 48 hrs of incubation period. The protease activity at 35^{O}C was found to be increased until 36 hours anddecreasedthereafter. The protease activity was found to be significantlyhighesthigherat 35^{o}C (99.20 U /g)onincomparison to 30^{o}C.

Key words. Tempeh, Acid protease, *Rhizopus oligosporus*, Fermentation, Soybean, Sorghum.

INTRODUCTION

In recent years, there is great difference in nutrition pattern and food preferences Nevertheless, interests for the consumption of the functional healthy foods have gained momentum due to consumer awareness. (Kaur and Das, 2011)Sorghum (*Sorghum bicolor*) known to us as Jowar, originated in Africa and has spread throughout the globe. Sorghum is a genus of about 25 species of flowering plants in the grass family Poaceae. Some of these species have grown as cereals for human consumption and some as pastures for animals. India ranks fifth in total sorghum production with 4.78 million tonnes grown in an area of 4.39 million hectares in 2020-21, Sorghum is generally consumed in Indiaas traditional food forms mainly as porridge in rural and socioeconomically weaker section. Sorghum is widely used as forage foranimals.Sorghum grain is a rich source of macronutrients (carbohydrates, proteins, and fat) and micronutrients (minerals and vitamins). It has about 70% carbohydrate, 3.5% fat and 11 % protein. One of the major impediments for its use as food is the lower availability of protein, starch, and minerals due to the presence of anti-nutritional factors like tannins and phytic acid (Kobue- Lekalake *et al*., 2007). However, processing methodlike fermentation has proven to reduce the anti-nutritional factors, thus improving the nutritional availability and the functional properties of sorghum. Consumption of tempeh has been rapidly increasing nowadays, in many countries. Tempeh could be prepared with cereals and pulses other than soybean thereby increasing the acceptability and digestibility of the grains. (Erkan *et al.* 2019).The intention of this study was to standardize the tempeh production at different temperatures from sorghumalone andin combination with soybean. The protease activity of the *Rhizopus oligosporus*culture was also studied.

MATERIALS AND METHODS,

Mataerials

Tempeh starter culture *Rhizopus oligosporus* MTCC 556strain was obtained from IMTECH. Chandigarh, the sorghum grains (*Sorghum bicolor*) and soybean (*Glycinemax*) used in the study was procured from local market in Coimbatore. Chemicals used for theresearch were of analytical grade.

Inoculum preparation

Preparation of *Rhizopus oligosporus* culture

The strain was grown on Potato dextrose agar (PDA) agar slant (Oxoid, Catalog No.???, UK) for 48 h at 25^{o}C. The culture was then preserved at 4^{o}C and further sub cultured on the PDA slant at the interval of 30 days to retain theviability. Inoculum was prepared by transferring a 10 ml of sterilized distilled water to48-hour old slant of *R.oligosporus*. The spores were taken under the sterileconditions and inoculum used in this study had a concentration of 10^{7} spores / ml(reference, ???).

Tempeh preparation

The tempeh was prepared in the laboratory according to the procedure described by (Steinkraus 1996,)as follows: First, sorghum and soybean were cleaned to remove dirt, stones, weed seeds, damaged grains and any other extraneous matter. The grains were taken as per the experiment treatments such as T1-Soybean(control), T2-Soybean +Sorghum (1;1).T3-Soybean +Sorghum (1:2), T4-Soybean +Sorghum (1:3) and T5–Sorghum. They were washed and soaked in clean tap water for 1 hr at room temperature (28°C), and then boiled for 30 minutesto partially hydrate the grains. The hulls were then removed by hand, The grains were then soaked in water overnight and the excess hulls were removed, subsequently the grains were boiled for 60 minutes to facilitate partial cooking. Following the precooking the grains were cooled to 37^{o}c and air dried prior to inoculation. Before inoculation of fungal spore suspension, the pH of the substrate was adjusted to 5.5 using acetic acid at 2.0 ml per 100 g of substrate to maintain uniformpH in all substrates andto facilitate the growth of fungi. The grains were inoculated with *R. oligosporus* MTCC 556 @ 0,05 g per 100 g of distributed grains. The samples were mixed thoroughly such that the mould spore was evenlydistributed over the surface of all grains. The grains were then packed in plastic bags perforated at 0,25 cm intervals and incubated at 30^{0}C and 35^{0}C and 40^{0}C,

pH measurement of fermented tempeh

The pH of the incubated tempehat different temperature was monitored during fermentation at every 12 hoursintervalsto study the pH changes and to determine the optimum fermentation time and temperature. The pH was measured at room temperature (20˚C -25℃) using a digital pH meter The pH meter was calibrated with buffer standards of pH 4.0 and pH7.0 before use. The measurements were taken in triplicate and average valueswas calculated

Protease assay

Crude enzyme extract

Tempeh (10 g) were mixed with 100 ml of 0.05 M phosphate buffer (pH 5) and homogenized.The homogenized mixture was kept at room temperature for 30 min with frequent stirring, and then centrifuged at 10000 rpm for 10 min. The supernatant was used as crude enzyme extract.

Protease activity

Protease activity was determined by the method of Yang and Huang (Yang SS and Huang CI, 1994). The reaction mixture containing 2 mL of 1 % casein solution in 0.05 M phosphate buffer (pH 5) and 1 mL of enzyme solution were incubated at 60^{o}Cfor 15 min and the reaction was then stopped with the addition of 3 mL of 10 % trichloroacetic acid. After 10 min the entire mixture was centrifuged at 10000 rpm for 10 min at 4^{o}Candthe absorbance of the liberated tyrosine in the filtrate was measured at 280 nm. One proteolytic unit (U) was defined as the amount of the enzyme that releases 1µg of tyrosine per

min under assay conditions.

Statistical analysis

Data were assessed by analysis of variance (ANOVA) using SPSS program version 16.0 and means were separated by Duncan's multiple range test with a probability ($p< 0.05$).

RESULTS AND DISCUSSION

Effect of temperature on Growth characteristics and duration of fermentation.

Growth of *R. oligosporus*on the substratesincubated at different temperatures isrepresented in fig1. The results indicated differenceamong temperature ranges (30, 35 and 40^{o}C). The favourable temperature for the growth of *R.oligosporus*was found to be 30 and 35^{o} C,while nogrowthwas observed at 40^{o}C indicating that 30 and 35^{o}Cwere found to be the optimum temperatures for fermentationof sorghum tempeh. The temperature within thefermenting beans mass does not raise above approximately 40^{o}C or the high temperature may damagesubsequent growth of the mould (Han *et al.,* 2001). Incubation temperature of 37^{o}C favoured the growth of*R. oligosporus* while being less favourable for growth ofthe mesophilic moulds and fewer bacterial species.(Wang *et al.,*1974). There was significant difference among the temperature on growth of *Rhizopusoligosporus.*The fermentation was significantly superior and quicker at 35^{O}C than30^{o}C in all treatments. Among the substrates T1(soybean 100%) and T2(Soybean+Sorghum 1:1) recorded less fermentation duration of 36 hrs while in T5(sorghum 100%)the duration for complete growth of the *Rhizopus*was found to be 38hrs at 35^{o}C. The fermentation duration ranged from 46 to 49 hrs at 30^{O}C as depicted in fig 1. Thebest temperature for tempeh production from sorghum alone and in combination with soybean was found to be 35^{o}C.This corroborated well with findings of Han *et al., (*2002) and Reddy *etal*., (2008).The optimum temperature of 35.8 ^{o}C was required for chickpea tempeh formation with *Rhizopus stolonifera*(Reyes-Moreno *et al*., 2000).

Changes in pH

Table 1 reveals theeffect of temperature on pH of the substrates during fermentation at different time intervals. Growth of the mould was accompanied by rapid increase in pH in all the treatments. The pH increased up to 48 hrs and remained constant thereafter at incubation temperature of 35^{o}C in all the treatments. At30^{o}C the pH increasedsteadily up to 60 hrs beyond which it remained constant.The increasein pH value is due to the significances of protein metabolism by the moulds.Muzdalifah *et al.*(2016) reported that the longer *tempeh* fermentation time, the pH increases exceeded pH 8.R.A. Sparringa,and J.D. Owens,1999 stated that pH increased to 6.6 -7.1 in matured tempeh as result of ammonia production.

Protease activity

The proteaseactivityduring tempeh fermentation of sorghum alone and in combination with soybean incubated at different time intervals arepresented in Table 2,Protease activity increased with increase in duration of fermentation up to 36 hrsbeyond whichit decreased significantly. The proteaseactivity was significantly highest at 35^{o}C when compared to30^{o}C.Protease activity was maximum andsignificantly higher inSoybean 100%(99.20 U /g)followed by Soybean+Sorghum (1::1) treatment (90.32 U /g). Least protease activity of 83.24 U/gwas observed in sorghum 100%. Muhammad Gul Sher *et al.* (2011)reported the maximum protease units (99.52 + 1.12 IU/g) in fermented barley at 36 hrs of incubation.

Conclusion.

The present preliminary study,demonstrated thattempehcould be developed fromsorghum alone and in combination with soybean. The optimum temperature for proliferative growth of*R.oligosporus* andmaximum protease production was standardised as 35^{O}C.Thus fermentation could open an avenue for better utilisation of neglected cereal like sorghum for human nutrition.

Table1: Effect of temperature on pH during tempeh fermentation at different time intervals.

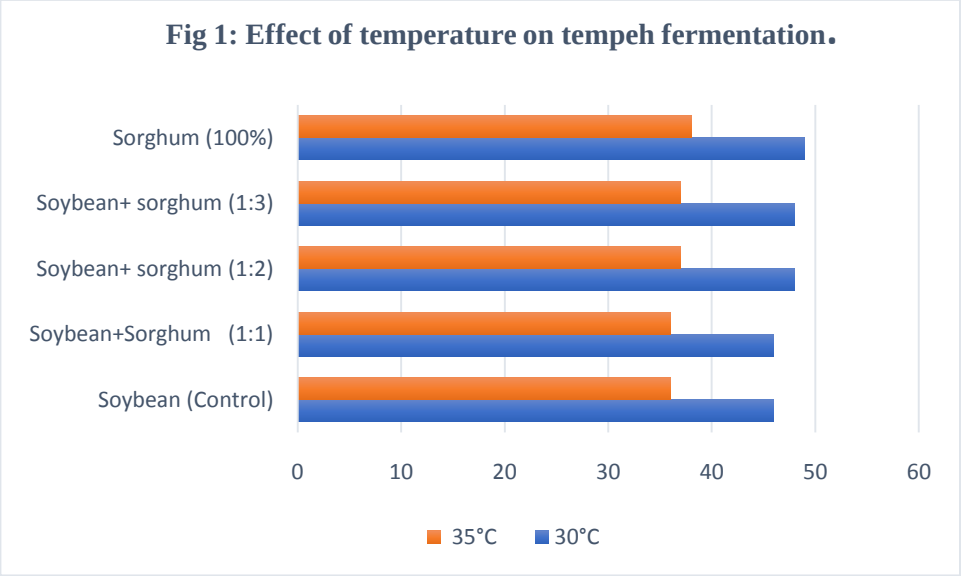
Treatments	Temperature ^{0}C	Fermentation Time (hrs)				
		12	24	36	48	60
Soybean (100%)	30	6.10 ± 0.02^{cA}	6.30 ± 0.06^{dB}	6.50 ± 0.04^{cC}	6.90 ± 0.02^{bD}	7.20 ± 0.02^{aE}
	35	6.10 ± 0.02^{bA}	6.35 ± 0.0^{cB}	6.60 ± 0.06^{eC}	7.20 ± 0.00^{cD}	-
Soybean+Sorghum (1:1)	30	5.90 ± 0.03^{aA}	$6,27\pm0.04^{bB}$	6.55 ± 0.02^{cC}	6.90 ± 0.02^{bD}	7.30 ± 0.04^{aE}
	35	6.00 ± 0.04^{bA}	6.38 ± 0.02^{eB}	6.55 ± 0.04^{cC}	7.20 ± 0.04^{cd}	-
Soybean+ Sorghum (1:2)	30	5.80 ± 0.04^{aA}	6.12 ± 0.04^{aB}	$6,38\pm0.02^{bC}$	6.90 ± 0.04^{bD}	7.30 ± 0.02^{aE}
	35	5.90 ± 0.04^{aA}	6.24 ± 0.04^{B}	6.50 ± 0.06^{cC}	7.25 ± 0.04^{cD}	-
Soybean+ Sorghum (1:3)	30	5.75 ± 0.04^{aA}	5.90 ± 0.02^{aB}	6.20 ± 0.04^{aC}	6.75 ± 0.03^{aD}	7.25 ± 0.02^{aE}
	35	5.80 ± 0.02^{aA}	6.10 ± 0.06^{aB}	6.55 ± 0.06^{cC}	7.20 ± 0.04^{cD}	-
Sorghum (100%)	30	5.75 ± 0.04^{aA}	6.20 ± 0.02^{bB}	$6,45\pm0.04^{bC}$	6.80 ± 0.04^{aD}	7.30 ± 0.02^{aE}
	35	$5,80\pm0.06^{aA}$	$6,25\pm0.04^{bB}$	6.50 ± 0.06^{cC}	7.25 ± 0.02^{cD}	-

Values are mean±standard deviations of triplicates.
Means with small alphabet superscripts within columns, and those with capital alphabet superscripts within rows are significantly different ($p < 0.05$).

Table 2: Effect of temperature on protease activity (IU/g) during fermentation at different time intervals.

Treatments	Temperature ^{0}C	Fermentation Time (hrs)				
		12	24	36	4	60
Soybean (100%)	30	7.04 ± 0.02^{iA}	76.08 ± 0.04^{gB}	92.65 ± 0.04^{fC}	93.20 ± 0.02^{gD}	8.54 ± 0.04^{dE}
	35	7.58 ± 0.04^{JA}	84.38 ± 0.02^{hB}	99.20 ± 0.04^{gC}	9.74 ± 0.04^{cD}	-
Soybean+Sorghum (1:1)	30	5.84 ± 0.02^{gA}	72.80 ± 0.04^{eB}	86.20 ± 0.04^{Dc}	88.27 ± 0.05^{fD}	4.60 ± 0.02^{c}
	35	6.34 ± 0.04^{hA}	75.10 ± 0.04^{fB}	90.32 ± 0.02^{ec}	8.26 ± 0.02^{bD}	-
Soybean+Sorghum (1:2)	30	4.08 ± 0.03^{dA}	69.50 ± 0.02^{cB}	80.90 ± 0.04^{bc}	82.34 ± 0.04^{eD}	4.80 ± 0.02^{cE}
	35	$4,78\pm0.02^{fA}$	73.20 ± 0.02^{eB}	85.50 ± 0.06^{dc}	8.20 ± 0.04^{bD}	4.40 ± 0.03^{bE}
Soybean+Sorghum (1:3)	30	$3,72\pm0.00^{cA}$	65.56 ± 0.03^{bB}	77.25 ± 0.05^{ac}	79.56 ± 0.04^{dD}	4.20 ± 0.00^{bE}
	35	4.53 ± 0.02^{eA}	$71,80\pm0.04^{dB}$	83.60 ± 0.05^{cc}	7.87 ± 0.04^{bD}	4.60 ± 0.02^{cE}
Sorghum (100%)	30	2.52 ± 0.04^{aA}	63.21 ± 0.04^{aB}	76.50 ± 0.04^{ac}	78.13 ± 0.02^{dD}	2.19 ± 0.04^{aE}
	35	2.86 ± 0.02^{bA}	$71,20\pm0.05^{dB}$	83.24 ± 0.06^{cc}	4.88 ± 0.04^{aD}	-

Values are mean±standard deviations of triplicates.Means with small alphabet superscripts within columns, and those with capital alphabet superscripts within rows are significantly different ($p < 0.05$).



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