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Full Length Research Paper

Process improvement as influenced by inoculum and product preservation in the production of Hawaijar - A traditional fermented Soybean

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Hawaijar is a fermented soybean product of Manipur state of India, which is traditionally prepared by fermenting cooked bean wrapped with Ficus (*Ficus hispida)* leaves in a bamboo basket. Microorganisms isolated from the traditionally fermented product were *Bacillus subtilis* and *Xanthomonas*. Individual and combined cultures indicated that *B. subtilis* would be the organism of choice based on physical and organoleptic attributes. Preservation of the fresh product with acetic and citric acid combinations gave better results than sodium chloride and acetic acid. All the products were evaluated on their organoleptic attributes. The oven-dried product found acceptance by the sensory panelists and was to be similar to the sun-dried product.

Key words: Hawaijar, *Bacillus subtilis*, preservation, ficus leaves, organoleptic evaluation.

INTRODUCTION

Traditional food systems are considered to be the backbone of modern food industries. In India, the fermented soybean product Hawaijar is traditionally consumed in Manipur (Irabanta and Umabati, 1995), which is incorporated in various curry preparations to add flavour and softness to recipes. It is also consumed with green chili and salt in the form of 'Chatani'. A non salted solid state traditional fermented soy food Kinema is also consumed in the eastern hills of Nepal, Darjeeling hills of West Bengal and Sikkim in India (Karki, 1986; Sarkar and Tamang, 1994; Shrestha and Noomhorn, 2001). Fermentation is one of the oldest and economical methods for producing and preserving food. By fermentation, the food may be made more nutritive, more digestible or impart better flavour.

Soybean (*Glycine max* L) is known to have a characteristic objectionable fishy or paint-like odour and anti nutritional factors like trypsin inhibitor. Consumption of raw soybean causes growth depression, pancreatic hypertrophy, hyperplasia and adenoma in experimental animals (Rackis, 1974; Yanatori and Fujita, 1976; Rackis and Gumbmann, 1981). On the other hand, fermented soybean has a wide range of health benefits like anti-cancer effects (Yee, 2000), reduction of menopausal syndrome frequency, osteoporosis protection (Anderson, 2000; Yee, 2000) and coronary heart disease prevention due to anti-atherogenic properties.

Traditional method of Hawaijar preparation involves soybean soaking for 12 to 24 h and cooking for 1 h till the soybean get cooked. After the excess water is drained out, it is wraped with healthy *Ficus hispida* leaves. The packed soybean is then kept in a bamboo basket covered with lid, which is lined with healthy clean banana leaves. Thereafter, the basket is kept under the sun for fermentation. The whole process takes about 4 to 5 days.

During fermentation light ammoniacal aroma is emitted and the bean turns into grayish tan coloured and sticky. The desired flavour and taste cannot be perceived if the boiled seeds are not wraped with these leaves (Irabanta and Umabati, 1995). The fermented soybean Hawaijar

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has a very short shelf life of 3 to 4 days after fermentation at room temperature. The present paper deals with the investigation of pure cultures inoculation effects on Hawaijar, its physico-chemical properties and shelf-life studies.

MATERIALS AND METHODS

Collection of samples

Soybean samples were collected from a local market of Manipur, India. Ficus *(F. hispida)* leaves were collected from Imphal region of Manipur.

Microbial analysis

Samples of traditional fermented Hawaijar (5 samples) and Ficus leaves (2 samples) were collected from various localities of Manipur. All samples were analysed on their bacterial population. 1 g of sample was homogenized with 90 ml of 0.85% (W/V) sterile physiological saline, vortexed for 1 min, and serially diluted in the same diluent. For the leaves sample, one gram of the washed sample was cut into small pieces with a sterile knife and suspended in saline and processed as described above Microbial growth was enumerated by plating serially diluted samples on trypticase soy agar and nutrient agar and incubating at 30°C for 2 days. Pure colonies were randomly selected from plates and analyzed by microscopic examination, Gram staining and identified based on biochemical methods. Selected bacterial strains were preserved in 15% (v/v) glycerol at –20°C.

Characterization and identification of the isolated bacterial strains

Characterization and identification of the isolated bacterial strains were carried out based on biochemical tests. Bacterial strains were identified according to Bergey's Manual of Systematic Bacteriology (Holt et al., 1994).

Preparation of Hawaijar

Soybean seeds were soaked for 12 to 24 h and cooked for 30 min till softened. Excess water was drained out and it was then independently inoculated with *B. subtilis, Xanthomonas* sp or their combination in different lots. Inocula were prepared by growing the cultures in nutrient broth, at 30°C on a rotary shaker (180 rpm). The culture broth with an OD of approximately 1.5 at 660 nm (approximately 10^6 CFU/ml) was used as inoculum at 5% (V/W). It was thoroughly mixed with the cooked beans. The inoculated beans were heaped inside bamboo baskets and incubated at 4, 27, 37, and 45°C respectively.

Samples of 250 g in duplicates were withdrawn for analysis. For control samples, cooked soybeans were wrapped with leaves of *F. hispida* and packed in bamboo baskets lined with fresh banana leaves. The baskets were kept under sunlight during the day on a raised platform, and kept inside the room at night. Fermentation proceeded for 4 days.

Analysis of proximate composition

Samples of fermented soybean (10 g) were blended with 20 ml of water in a homogenizer for 1 min and the pH of the slurry was directly determined using a digital pH meter. Fat, crude fiber, reducing sugar, total carbohydrate, moisture, ash and total crude protein were analysed according to standard methods (AOAC, 1998). All samples were analysed in duplicate.

Organoleptic evaluation

Samples were subjected to organoleptic evaluation without the addition of any taste or odour enhancers like salt or spices. 6 panelists (4 male and 2 female panelists in the age group of 18 to 30) who are regular consumers of the product were provided with coded products to taste. Organoleptic evaluation was performed based on a 10- point Hedonic scale. Attributes like appearance, texture, flavour taste and overall acceptability were tested. Dried samples were rehydrated with sterile water for 15 min before analysis.

Preservation experiment

Combinations of acetic acid (0.2%)-citric acid (0.5%) (v/v) and sodium chloride (0.5%)-acetic acid (0.2%) (w/v) in equal volumes were used. Product was steeped in the solution for 5 min, excess liquid was drained out, and sample was air dried on blotting paper.

Drying

Oven drying

Samples (25 g) were made as lumps of approximately 5 cm diameter and dried in a drier at 75°C for 3 days till the samples were dried to a moisture percentage of 12.

Sun drying

Samples (25 g) were made as lumps as described above, and dried under sunlight for 8 days with an exposure of 6 to 8 h a day till the samples were dried to a moisture percentage of 12. All the samples were packed in an air tight container and stored at room temperature.

RESULTS AND DISCUSSION

Microorganism

Two morphologically different isolates which predominated in all the tested samples were isolated and identified based on their morphological and biochemical features as *B. subtilis* and *Xanthomonas* sp. Analysis of the isolated colonies revealed that *B. subtilis* was prevalent (70%) and the remaining isolates belonged to *Xanthomonas sp*. Irabanta and Umabathi (1995) earlier reported the presence of these two microorganisms in Hawaijar.

Physicochemical changes of the fresh product

Fermentation was carried out after inoculating with *B. subtilis*, *Xanthomonas* sp. and their combinations, along

Table 1. Physical changes of soybean during Hawaijar production under different fermentation conditions

Sample incubation	Temperature°C	Colour	Stickiness	Flavour fermentation	Time (days)
	4	Yellow	--	Beany	9
Wrapped	22	Light tan	+	Low flavour	5
With *Ficus*	37	Tan	++++	Very good flavour	2
Hispada leaves	45	Tan	++	Good flavour	2
Bacillus subtilis	4	Yellow	--	Beany flavour	9
	22	Light tan	+	Low flavour	5
	37	Tan	++++	Very good flavour	2
	45	Tan	++	Good flavour	2
	4	Yellow	--	Beany flavour	9
Xanthomonas sp.	22	Yellow	--	Beany flavour	5
	37	Yellow	+++	Very low flavour	2
	45	Yellow	+	Very low flavour	2

-- Not sticky, + Slightly sticky, ++ Less sticky, +++ Sticky, ++++ Very sticky

Table 2. Proximate composition of soybean and *Hawaija.*

Parameters (%w/w)	Soybean	Hawaijar*
Total crude protein	35	43.8
Total fat	4.75	1.75
Total carbohydrate	28.6	9.4
Reducing sugar	1.10	3.1
Total ash	3.99	3.88
Total crude fiber	3.51	5.56
Moisture	10.4	13.8
pH	6.0	8.6

* Inoculum: *Bacillus subtilis*

with controls at various temperatures in bamboo baskets. The Hawaijar product obtained was moist, sticky, tan coloured and with ammoniacal odour. Traditionally Hawaijar is prepared by wraping sprouted, boiled soybean in ficus leaves which acts as inoculum, along with thermotolerant organisms from soybean, which survive the boiling step. Present results (Table 1) indicate that samples fermented with *B. subtilis* and incubated at 37°C had acceptable quality similar to the traditional fermented product. Although the traditional product varies in quality and time of incubation based on the season of preparation, due to temperature changes from freezing to 40°C, present results indicated that 37°C would be ideal for the fermentation under study and for the time required to complete the fermentation (2 days).

Proximate analysis

The proximate composition of the product indicated that protein increased along with crude fiber content due to fermentation, whereas fat and total carbohydrate contents decreased most likely due to their utilization by the growing microorganisms (Table 2). The increase in reducing sugars would result from the breakdown of complex carbohydrates into simple sugars. The end fermented product exhibited an alkaline pH. The increase in pH during fermentaion may be related to microbial activity like proteolysis. It has been suggested that liberated ammonia or other basic end-products from protein decomposition may be the cause for the development of alkaline pH (Ikenebomeh et al., 1986). Odufa (1981) also observed a rise in pH in the fermentation of African locust bean to dawadawa.

During fermentation, starch and other complex sugars in the beans get hydrolysed to easily utilizable sugars. However, the most significant biochemical change that occurs during Hawaijar fermentation is protein hydrolysis. This is due to the high proteinase activity which results in a rapid amino acid production. Such observation were made earlier while working with the fermentation of Japanese miso (Schibasaki and Hesseltine, 1962; Steinkraus et al., 1965), Nigerian ogri (Odufa, 1981) and Soya dawadawa, (Dike and Odufa, 2001) who, reported

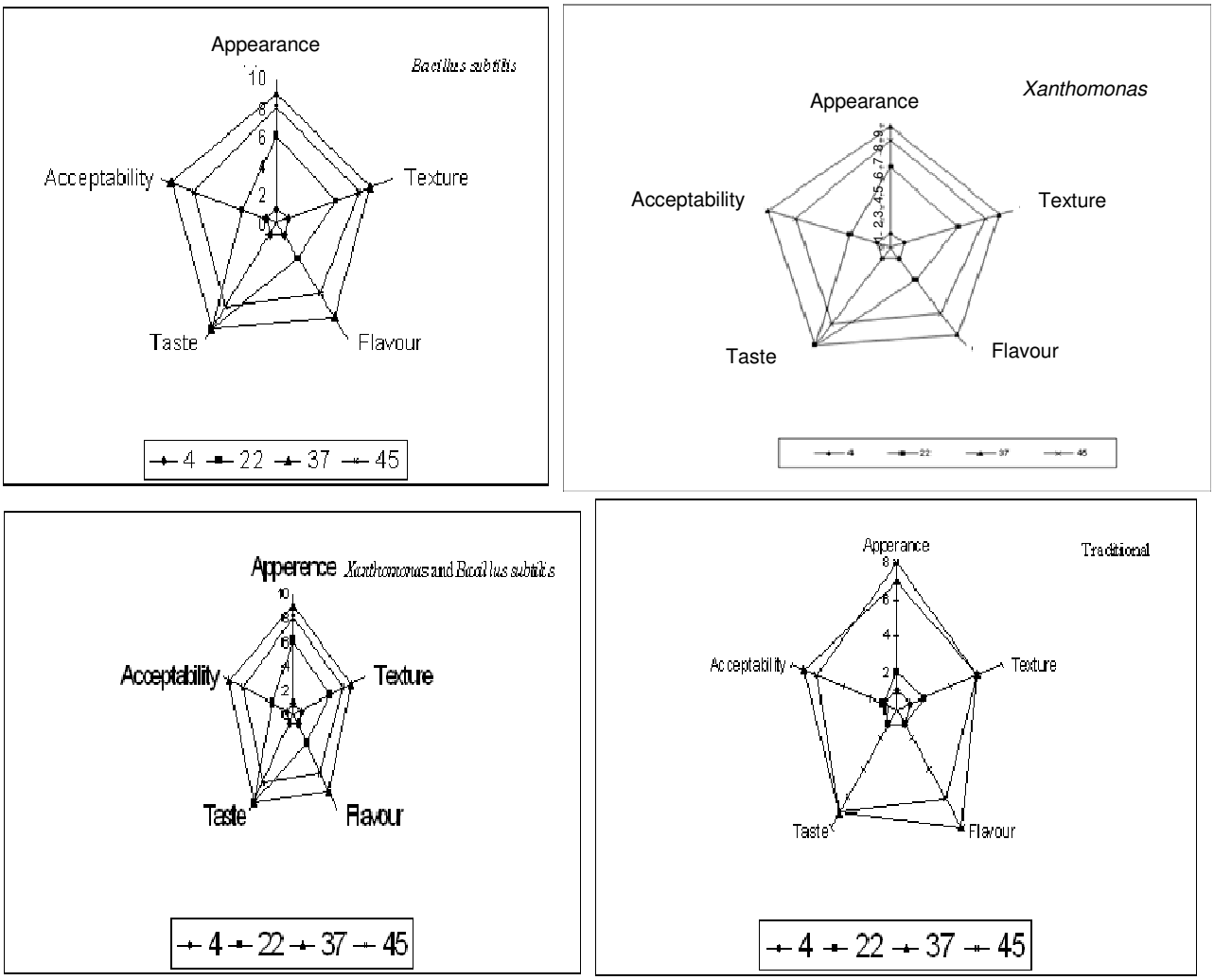

Figure 1. Sensory evaluation of Hawaijar prepared with different inocula (inoculum used at 0.5% of ~1.5 OD culture at 660 nm) and incubated at various temperatures (4, 22, 37 and 45°C).

high protease activity, which, in turn increased the amino acid content of the product. The increase in amino acid content with fermentation time is especially important from the nutritional point of view as it would increase digestibility and absorption. With respect to the carbohydrates, soaking, germination, heating and roasting normally decrease the total carbohydrate contents.

This has been related to the active respiration taking place during soaking and germination (Rackis and Gumbmann, 1981). Soaking, germination and heating also increased reducing, non-reducing and total sugars due to α-and β-amylases which increase with soaking.

Organoleptic evaluation

Organoleptic evaluation of the fermented Hawaijar indicated that the product produced at 37°C was highly acceptable irrespective of the inoculum used (Figure 1). The products obtained with different inocula were tested for sensory acceptability. The product prepared by using *B. subtilis* scored better than the traditional product, followed by the combination of Xanthomonas sp. and *B. subtilis*, whilst the one from *Xanthomonas* sp. alone exhibited the lowest score.

Preservation

The high moisture content imparts the product a short shelf-life of 3 to 4 days at room temperature. Therefore, Hawaijar is normally preserved after drying. The traditional method involves drying the product under the sun. Results in Table 3 indicate that panelists accepted both solar-dried and oven-dried samples. Both products were classified as similar in colour, odour and texture when evaluated by the sensory panelists. Traditionally, dried

Table 3. Effect of the drying method on sensory and physical properties of Hawaijar after 9 days of preservation*

Method	Colour	Odour	Texture
Sun-dried (7 days)	Change in tan colour to dark brown	No change	No change
Oven-dried (45^{o}C, 3 days)	Change in tan colour to dark brown	No change	No change
Control (not dried)	Change in tan colour to dark brown	Putrified	Not acceptable

* Inoculum: *Bacillus subtilis.*

Table 4. Sensory evaluation scores for solar- and oven-dried Hawaijar*

Method	Colour	Appearance	Texture	Flavour	Taste	Overall acceptability
Solar-dried	Change in tan colour to dark brown	7	6	7	7	7
Oven-dried	Change in tan colour to dark brown	7	5	6	7	7
Control	Change in tan colour to black	2	2	1	0	0

* Inoculum: *Bacillus subtilis.*

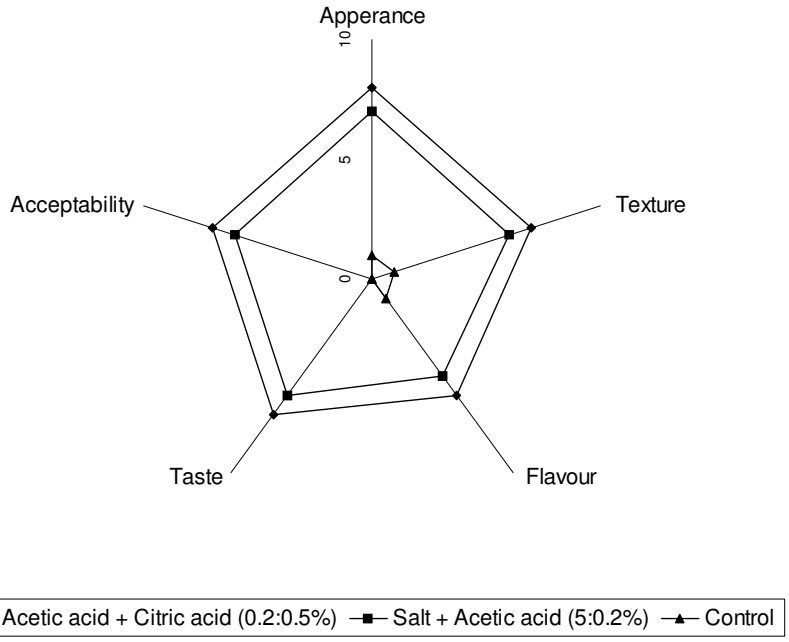

Figure 2. Sensory evaluation of preserved Hawaijar with various preservatives (after 7 days of storage at room temperature in an air tight container).

products are preserved for more than a month. As the change in the dried product quality occurs in the first 5 to 7 days, the study was extended for 9 days only. Sensory attributes of oven-dried Hawaijar were equally rated to those of the sun-dried product (Table 4), showing a similar profile as judged by the panelists. As the control sample was spoilt, the panelists did not taste that product. Additionally, an important requirement to commercialize the product is to increase its shelf-life.

Various natural preservatives like acetic acid, citric acid and salt were used. Combinations of acetic acid (0.2%) with citric acid (0.5%) or sodium chloride (0.5%) increased the shelf-life of the product by 7 days (Figure 2). There was no change in the colour and odour in all treatments and the product was acceptable till the end of the storage period. According to sensory attributes (Table 5), the combination of acetic and citric acids was more acceptable than salt plus acetic acid. This may be due to the neutralization of the basic pH of the product by the organic acids. The salt thus formed might have imparted a better taste to the product along with its preservative effects.

The herein presented results indicated that the production method of both fresh and dry Hawaijar can be

Table 5. Effect of preservatives on the shelf-life of Hawaijar*

Preservative	Colour	Odour	Shelf-life (days)
Acetic acid + citric acid (0.2:0.5%v/w)	No change	Good	7
Salt + acetic acid (0.5:0.2% w/v)	No change	Good	7
Control	Darker colour	Not acceptable	3

* Inoculum: *Bacillus subtilis.*

standardized by selecting appropriate inocula and fermentation conditions and that the process can be successfully carried out with *B. subtilis* inoculum. The proposed methodology led to similar results on the product at the same incubation time. Preservation with common salt and acetic acid could improve the shelf life without affecting sensory properties. Drying can be carried out in a hygienic condition with in shorter period in a dryer. The present results could help the production of Hawaijar to be produced as cottage industry product.

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