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Comparative biochemical analyses of soybean seeds, *hawaijar* and stored *hawaijar*

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Hawaijar, a naturally fermented indigenous soybean food, plays a very importnat role in food habits of the people of Manipur. In this paper, the biochemical properties of the raw soybean seeds, *hawaijar* and stored *hawaijar* are compared. The pH of fermented soybean increased with fermentation time (7.49 on the third day) and days of torage. The mean moisture content of raw soybean seeds was 9.97%, highest in bioled soybean seeds (63.85%) and reduced with subsequent fermentation and storage. Ash content did not show much change during the processing of soybean for *hawaijar* formation. Crude fibre content was lowest in boiled unfermented beans and highest in the three days fermented soybean. The percentage of soluble protein in boiled soybean seeds was lesser than the raw seeds which showed slight increase during fermentation and storage. The amount of free amino acids increased suddenly on the 3rd day of fermentation and slightly increased during storage. The fat content of boiled soybean seed was lesser than the raw unfermented seeds but increased suddenly during the 2nd day of fermentaion. The content of total soluble sugar of raw soybean seed (2.27%) was found to decrease to 1.2% after boiling. The reducing sugar level decreased during the processing of soybean for *hawaijar* production but increased slightly during fermentation.

Key words: *Hawaijar, Bacillus,* soybean, fermentation, biochemical changes

INTRODUCTION

During the past several decades, soybean [*Glycine max* (L.) Merrill] has become an increasingly important agricultural commodity, with a steady increase in annual production. Soybean has been well-recognized as an excellent source of high-quality soluble protein, lipid and minerals (Fonseca and Ward, 2004). Soybean is a good source of dietary fibres which accelerate excretion of harmful chemical compounds and regulate the function of the intestines.

Traditional soybean foods have been popular and consumed in Asia for many centuries. Soybean seeds are cooked and fermented by pure cultures of *Bacillus subtilis*, as for *natto* in northern Japan (Ohta, 1986), or fermented using contaminant natural *Bacillus* species, as for *dawadawa/iru* in west and central Africa (Campbell-Platt, 1980), *kinema* in Nepal, Sikkim and Darjeeling (Sarkar *et al*., 1994), *thua nao* in northern Thailand (Sundhagul *et al*., 1972) and *in- si (tou-si)* in China (Hesseltine, 1989).

In Manipur, *hawaijar* has been consumed as a regular food in every household. It is either consumed directly or used as a flavouring agent in vegetable items throughout the year and its addition makes the vegetable items more soft and tasty. It is used to prepare a very special dish known as *'chagempomba'* (Premarani and Chhetry, 2008, 2009) or as a paste with chilli and fresh vegetables *'Ametpa'* in Manipur (Jeyaram *et al*., 2008). The present study has aimed at comparing the biochemical properties of the raw soybean seeds the fermented product and the fermented and stored *hawaijar* in order to understand the various biochemical changes occurring during fermentation as well as during storage.

MATERIALS AND METHODS

Sampling: Soybean seeds of local variety [*Glycine max* (L.)Merrill] were purchased from *Ima* market, Manipur, during March 2005 and brought to the laboratory in sterile polythene bags for use in the whole period of investigation. In order to study the biochemical changes, hawaijar was prepared in the laboratory following the traditional method. Since the shelf-life of *hawaijar* is very short (4- 5 days), biochemical changes were analysed until 4th day of storage at ambient temperature. Samplings were collected at every 1 day interval.

Traditional method of preparation: In the traditional method of preparation, the soybean seeds were soaked overnight and washed well with tap water. They were then boiled well in a pressure cooker till the seeds became soft, washed with hot water, wrapped with a clean cotton cloth and packed tightly in a small bamboo basket with lid and a base, called *'lubak'*. The box was then wrapped with a jute cloth and kept in the sun for maintaining the high temperature required for *hawaijar* formation. The palatable stage of fermented soybean was noticed in 3-5 days. After fermentation, the soybeans developed a brown colour, a sticky texture and a distinctive fermented odour and taste.

Biochemical investigations: Proximate analysis was carried out on the unfermented soybean, boiled soybean, soybean at different stages of fermentation and the fermented and stored *hawaijar*, 2 g fermenting beans (wet wt.) were homogenized with 8 ml of distilled water and the pH of the slurry was measured with a Systronics type 335 pH meter (AOAC, 1990). Moisture content was calculated by drying the sample at 130± 1°C to constant weight (AOAC, 1990). Ash content was measured by heating the sample at 600°C until the difference between two successive weighing was <1mg. Crude fibre was also estimated (AOAC, 1990).

The quantity of soluble protein was determined by colorimetric method introduced by Lowry *et al.* (1951) with slight modification. The estimation of total free amino acid was done following the Ninhydrin method of Moore and Stein (1948). Fat was estimated by the method of A.O.A.C. with slight modification mentioned by Hart and Fisher (1971).

For the estimation of total soluble sugar, 1 g of the powdered unfermented soybean seed, boiled soybean and soybean at different stages of fermentation and storage was extracted with 80% ethanol by using mortar and pestle. The supernatant collected after centrifugation was then evaporated until it was free from alcohol. The extract was then made aqueous with a few ml of water. The amount of total soluble sugar present in the extract was determined by anthrone reagent (Dubois *et al.,* 1951).

RESULTS AND DISCUSSION

The prepartion of *hawaijar* was very simple and similar to Japanese *'itihiki- natto'* where whole soybeans were used for fermentation (Ohta, 1986). Unlike *'natto', 'hawaijar'* was prepared in bamboo baskets layered with *F.hispida* leaves or a cotton cloth. There was no addition of fire wood ash during *'hawaijar'* production like *'kinema'* (another Indian fermented soybean). Traditional *'hawaijar'* was characterized by its alkalinity, stickiness and pungent ammonia odour.

Biochemical analysis of soybean seeds, *hawaijar* and stored *hawaijar* showed that the pH (6.63) of raw soybean seeds was lesser than boiled seeds (pH 6.68) (Table 1). The pH of fermented soybean increased with fermentation time (pH 7.49 on the third day) and days of storage (pH 7.71 on the 1st day of storage to 7.91 on the 4thday). The observed pH trend is in accordance with the reports of Popoola et al. (2007). Increasing pH during fermentation has been attributed to proteolytic activities and the release of ammonia following the utilization of amino acids by microorganisms involved in the fermentation. The released ammonia is mainly responsible for the pungent smell that usually accompanies most vegetative protein fermentation (Sarkar et al, 1993). The mean moisture content of raw soybean seeds was 9.97% whereas it was highest in boiled soybean seeds (63.85%). As the soybeans were soaked and cooked prior to fermentation, *hawaijar* had high moisture content. With subsequent fermentation and storage, the moisture content reduced to 41.09% on the 4thday of storage (Table 1). This was because the fermentation occurred at a high temperature. Ash content did not show much change during the processing of soybean for hawaijar formation (Table 1). Crude fibre content of raw soybean was 3.8%, lowest in boiled unfermented beans (2.5%), highest in the three days fermented hawaijar (8.2%) and then decreased during the storage period (Table 1) .

Sarkar *et al.* (1994) and Jeff-Agboola and Oguntuase (2006) also reported an increase in the crude fibre content of soybean seeds after fermentation with *Bacillus* sp.

The percentage of soluble protein in boiled soybean seeds was lesser than the raw seeds. *Hawaijar* contained 26.02% soluble protein after three days of fermentation and showed slight increase during storage (Table 1). Jeff-Agboola and Oguntuase (2006) also reported an increase in soluble protein content after fermentation of soybean seeds. Microorganisms had been reported to increase the soluble protein content of samples on which they grow (Raimbault, 1998). Sarkar *et al.* (1994) also reported higher soluble protein content in the fermented soybean than the substrate.

Parameters[a]	Raw seed	Boiled seed	Fermentation period			Storage period			
			1stD	2ndD	3rdD	1stD	2ndD	3rdD	4thD
pH	6.63±0.02	6.68±0.00	6.74±0.01	7.06±0.02	7.49±0.01	7.71±0.01	7.83±0.03	7.86±0.02	7.91±0.01
Moisture (%)	9.97±0.12	63.85±0.04	63.8±0.02	63.75±0.11	62.1±0.02	57.05±0.02	54.7±0.20	47.14±0.04	41.09±0.01
Crude Fibre (% DM[b])	3.80±0.03	2.5±0.01	4.6±0.20	6.70±0.06	8.20±0.04	7.10±0.01	6.40±0.03	6.20±0.04	5.50±0.21
Soluble Protein (%DM[b])	24.5±0.05	17.70±0.02	18.02±0.20	25.50±0.06	26.02±0.02	27.03±0.04	27.20±0.06	27.35±0.10	27.60±0.02
Free amino acid (% DM[b])	0.82±0.01	0.77±0.03	1.02±0.03	2.0±0.02	3.80±0.07	4.30±0.43	4.53±1.02	4.08±0.10	5.03±0.20
Fat (%DM[b]) (ether extract)	20.0±0.12	16.5±0.11	18.13±0.12	22.53±0.31	24.36±0.06	25.5±0.02	26.0±0.21	26.2±0.01	26.20±0.10
Total soluble sugar (%DM[b])	2.27±0.03	1.20±0.11	0.20±0.04	0.80±0.02	0.90±0.04	0.92±0.07	0.95±0.03	1.10±0.02	1.32±0.04
Reducing sugar (%DM[b])	0.25±0.02	0.20=04	0.15±0.02	0.21±0.03	0.23±0.04	0.27±0.02	0.31±0.03	0.30±0.04	0.29±0.05
Ash (%DM[b])	0.97±0.04	0.95±0.02	0.98±0.02	1.20±0.11	1.42±0.06	1.47±0.04	1.5±0.09	1.42±0.04	1.32±0.02

[a]Values are means±SE of three replicate fermentations
[b]DM, dry matter basis

The amount of free amino acids increased suddenly on the 3rd day of fermentation (2% on the 2nd day to 3.80% on the 3rd day) and slightly increased during storage (Table 1). The rapid increase in total free amino acids in the early stages of fermentation was an indication of rapid protein hydrolysis. Proteolysis plays an important role during the ripening of *hawaijar* as in the case of other fermented soybean foods (Han *et al*, 2002; Agrahar-Murugkar and Subbulakshmi, 2006). Omafuvbe *et al.* (2002) also reported an increase in free amino acid content during the fermentation of soybean for soy-*daddawa* production. A similar increase in the level of free amino acids with fermentation has been reported (Jian- Hua *et al*, 2007; Omafuvbe, 2008). In most fermented high- protein products, the extent of protein hydrolysis is one of the most important factors for changes in texture and flavour (Whitaker, 1978). Soluble low molecular weight peptides and amino acids that contribute to flavour are produced through the enzymatic breakdown of proteins (Njoku and Okemadu, 1989; Ogbona *et al*, 2001).

The fat content of boiled soybean seed was lesser than the raw unfermented seeds. The amount of fat increased suddenly during the 2nd day of fermentation and continued to increase during the storage period (Table 1). This result is in agreement with the result obtained by Mbajunwa (1995) and Achinewhy (1983) and suggested that fermentation probably enhances oil extraction. Akindumila and Glatz (1998) reported that certain microorganisms could produce microbial oil during growth on substrate.

The content of total soluble sugar of raw soybean seed (2.27%) was found to decrease to 1.2% after boiling and kept on decreasing till the 1st day of fermentation (0.20%) (Table 1). Soaking and cooking resulted in partial loss of oligosaccharides for *natto* production (Kanno *et al*, 1982). These patterns of change in soluble sugar level have been reported in similar fermented condiments (Ogunshe *et al*, 2007). *Bacillus* species have been reported as producers of certain enzymes such as amylase, galactanase, galactosidase and fructofuranosidase, which are involved in the degradation of carbohydrates (Aderibigbe and Odunfa, 1990; Kiers *et al*, 2000).

The reducing sugar level decreased during the processing of soybean for *hawaijar* production but increased slightly during fermentation. Till the 2nd day of storage, the reducing sugar level increased which later decreased (Table 1). Similar changes were reported by Ogunshe *et al.* (2007). The increased level of reducing sugar is a reflection of the activities of α-amylase and sucrose in the fermenting seeds (Omafuvbe *et al.*, 2000). Microbial amylases hydrolyse carbohydrates into sugars, which are then readily digestible by humans.

Despite the awareness of science in the society, the production of *hawaijar* still remains the traditional family art practised in homes with rudimentary utensils. *Hawaijar* if properly developed have a strong potential of increasing food production, improving the nutritional status of the rural population and provide income to rural masses of Manipur. More research work is required on the components which are responsible for flavour, aroma and texture for optimization and large scale production of *hawaijar* and building consumers' confidence on the fermented food product.

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