

Research  
Paper

## Traditional fermentation of mustard green (*Brassica juncea*) enhances the nutritive value

KANANBALA SARANGTHEM AND HOIKHOKIM

See end of the paper for authors' affiliations

Correspondence to :

**KANANBALA SARANGTHEM**  
Department of Life Sciences,  
Plant Physiology Section,  
Manipur University,  
IMPHAL (MANIPUR)  
INDIA  
Email : kananbala\_s@rediffmail.com

### ABSTRACT

The leaf mustard plant or mustard green scientifically known as *Brassica juncea* (Cruciferae) are widely used as vegetables having high nutritive value and a good source of natural antioxidants. Fermented foods are popular throughout the world and in Manipur make a significant contribution to the diet of many individuals. In the study, effect of the traditional fermentation processes of mustard green on the nutritive value were assessed and found that the protein, flavonoid, tannins and phenolic contents increases after fermentation though carbohydrates, ascorbic acid decreases. The antioxidant properties of methanolic extract from fresh and fermented products were also assessed and it was found that the percent inhibition was higher in the fermented products (76.81% in 60 m/ml) against fresh leaves (66.30% in 60 m/ml)

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**KEY WORDS :** Mustard leaves, Fermented, Nutritive

The leaf mustard plant or mustard green scientifically known as *Brassica juncea* belongs to the family Cruciferae. They are widely used as vegetable throughout the world. Cruciferous vegetables are high nutritive value and a good source of natural antioxidants due to high level of carotenoids, tocopherols and ascorbic acid (Heinon *et al.*, 1989). Two special glucosinolates sinigrin and gluconasturian which can be converted into allyl-isothiocyanate and phenethyl-isothiocyanate a well documented cancer preventive are present in mustard green ([www.http// wikipedia.com](http://wikipedia.com)).

Mustard green are available surplus during the peak season (December - April) and they cannot be preserved longer in the fresh form due to the rapid decay cause by microbial action. So, in Manipur preservation of mustard green is done through traditional fermentation locally known as (Ankam thu). In addition to preservation fermented foods provides the bio nutrients minerals and fortified with bio active compounds enhancing the flavor and aroma and exert health promoting beneficial (Darby, 1979, Cambell-Platt, 1994, Steinkraus, 1998). Microbial fermentation resulted in significant variations in bio chemical contents (Sucheta and Chhetry, 2003).

Therefore the present work was carried out to study the effect of fermentation on the nutritive value of mustard green.

### RESEARCH PROCEDURE

Fresh mustard leaves were pluck from the garden, washed thoroughly under tap water; sun dried and then kept in an oven at 40°C for 12 hrs. The dried leaves were grounded into fine powder form and passed through a sieve and kept for analysis of biochemical contents.

#### Traditional methods of fermentation:

Fresh mustard leaves were pluck from garden in bulked, clean and spread over bamboo mat (locally called pheh) in the sun. The wilted leaves were crushed using traditional wooden crusher. The juice was extracted by squeezing with hand, and then boiled for ten minutes to slurry. It was stored in a hollow bamboo container for year long as reserved fermented food.

#### Estimation of total phenolic content:

Phenolic contents were estimated by the method of Donald *et al.* (2001). A known weight of fresh and fermented samples were extracted in methanol by intermittent maceration upto 48 hrs, centrifuge and the supernatants were used for the estimations. Chlorogenic acid was used as the standard and absorbance was measured at 765nm.

**Estimation of flavonoids :**

Aluminum chloride spectrophotometric method was used for flavonoid determinations (Chang *et al.*, 2002). The reaction mixture comprised of 0.1 ml of the extract, 0.1 ml of aluminium chloride (10%), 0.1 ml of potassium acetate (1 M) and 2.7 ml of distilled water. It was kept at room temperature (27°C) for 30 minutes and the absorbance was measured at 415 nm using quercetin as the standard.

**Estimation of carbohydrates (Anthrone methods):**

The sample was extracted in 80% alcohol and centrifuge. The supernatant was used for analysis. 1 ml of the supernatant was mixed with 4 ml of Anthrone reagent (2 per cent in conc. H<sub>2</sub>SO<sub>4</sub>). The absorbance was taken at 620 nm. Standard curve was prepared using glucose.

**Estimation of tannin:**

Tannin content was determined by Folin-Denis method (by Sadasivam and Manickam, 1992) which is based on the non-stoichiometric oxidation of the molecule containing a phenol hydroxyl group. Tannin like compounds reduced phosphotungstomolybdic acid in alkaline sodium carbonate solutions to produce highly blue coloured solutions. The intensity of which is proportional to the amount of tannin. The absorbance was measured at 700nm using tannic acid as the standard compound.

**Estimation of ascorbic acid:**

For the estimation of ascorbic acid contents volumetric method was used (Sadasivam and Manickam, 1992). The sample was extracted in 4 per cent oxalic acid, and centrifuge. The supernatant (ml) was filtrated against the standard 2,6 dichlorophenol indophenol reagent till the solution become pink.

$$\text{Ascorbic acid/100 g in mg of tissue} = \frac{I \times S \times D}{A} \times \frac{100}{w}$$

I= ml of indophenol reagent used in the titration.

S = mg of ascorbic acid reacting with 1 ml of reagent

D = Volume of the extract in ml.

A = The Aliquot titrated in ml.

W = Weight of the sample.

**Antioxidant potentialities:**

Determination of antioxidant activity by DPPH Scavenging assay (Fogliano *et al.* (1999) was adopted.

**Preparation of the extracts:**

1 g each of mustard leaves powder and its fermented form were separately extracted in 25 ml of Methanol at

room temperature for three days. Each extract was filtered and the filtrated were dried under room temperature. The extracts (0.008g) were dissolved separately in 10 ml of methanol and various concentrations (60, 40, 20, 10 big) were prepared. Each of the 2.5 ml test extract was mixed with DPPH (0.002g/10ml) and allowed 30 minutes for the reaction to occur. The absorbance of the colour developed was measured at 517 nm by a spectrophotometer. The negative control and positive control were also subjected to the same procedure. Three replicates were used and the average absorption was noted for each concentration. Per cent inhibition of DPPH was calculated by following equation (Lee *et al.*, 1998).

$$\% \text{ Inhibition} = 1 - \left( \frac{A_1}{A_2} \right) \times 100$$

where, A<sub>1</sub> is the absorbance of the test samples and A<sub>2</sub> the absorbance of control reaction.

**RESEARCH ANALYSIS AND REASONING**

Table 1 depicts the changes in biochemical contents. The protein, flavonoid, tannins and phenol contents increases after fermentation and carbohydrates, ascorbic acid decreases. This may be due to microbial action (Woston *et al.*, 1991 and Muller *et al.*, 2000) observed that protein are found to occurred at the tip region of filamentous fungi and thus led to the high content of protein in the fermented product. Bellmer (1977) showed the formation of phenolic compound by bacteria help in flavour production and act as a defense mechanism against unwanted bacteria. The important bio chemical contents such as ascorbic acid, vitamin C and carbohydrates contents decreases in fermented product. Similar to our present findings, Adam and Moss (1996) reported that during Sauekra fermentation several vitamins were partially conserved particularly ascorbic acid and vitamin C and a consistent relationship between per cent loss of carbohydrates and increases of protein contents in solid substrate fermentation of potato waste by Maninder *et*

**Table 1: Biochemical contents of fresh and fermented mustard leaves**

Parameters	Fresh mustard	Fermented mustard
Flavonoids	1.29mg/g ±0.27	1.68mg/g ±0.41
Proteins	4.32mg/g ±0.13	7.88 mg/g ±0.62
Tannins	0.22mg/g ±0.31	3097 mg/g ±0.78
Phenols	18.32mg/g ±0.15	27.57 mg/g ±0.84
Carbohydrate	2.104 mg/g ±0.46	1.08 mg/g ±0.72
Ascorbic acid	3.54 mg/g ±0.45	1.43 mg/g ±0.67

Standard error of the mean (n=3).

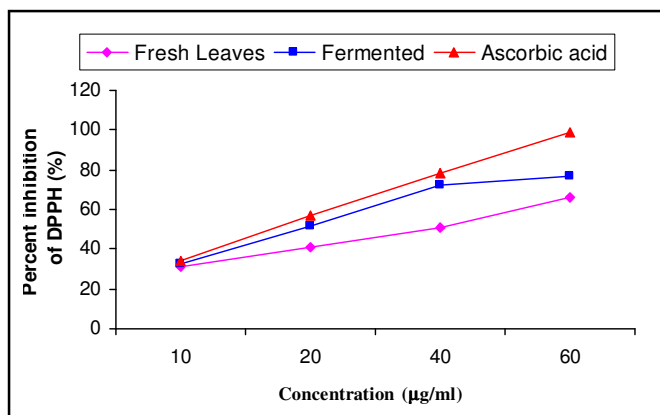


Fig. 1 : Per cent inhibition of DPPH by fresh and fermented mustard leaves extract

al. (2000). Dahal *et al.* (2003) reported that during masyaura a fermented savory of Nepal found that an increase in soluble protein, amino acid, nitrogen and vitamin B complex and decrease in pH, starch, free sugar and reducing sugar.

Table 2 depicts the antioxidant properties of methanolic extract from fresh and fermented sample, it was found that the per cent inhibition is higher in the fermented products (76.81% in 60 m/ml) against fresh leaves (66.30% in 60 m/ml) the higher percentage inhibition in the fermented form might be attributed to the high contents of phenolic compounds (*i.e.* phenol, flavonoid and

Table 2 : Per cent inhibition of DPPH by fresh and fermented mustard leaves extract

Materials	% inhibition of DPPH (µg/ml)			
	10	20	40	60
Fresh leaves	30.87	40.76	50.8	66.30
Fermented	32.55	51.92	72.28	76.81
Ascorbic acid (Standard)	34.2	56.94	78.51	98.63

tannin) as phenolic compounds can act as antioxidant by radical scavenging in which they break the free radical chain through hydrogen atom donation (Kosem *et al.*, 2007). The relationship between phenol and flavonoid contents with antioxidant activities was reported by many authors (Pourmorad *et al.*, 2006, Keshavkant *et al.*, 2008, LimYau Yan *et al.*, 2006). Hence, it is concluded from our findings that the nutritive components such as protein, phenols, flavonoid and tannin were enhanced in the fermented product which were the components of antioxidants properties. Therefore, surplus mustard leaves during peak season should be preserved through fermentation, and wastage should be avoided for off-seasonal uses.

#### Authors' affiliations:

**HOIKHOKIM**, Department of Life Sciences, Plant Physiology Section, Manipur University, IMPHAL (MANIPUR) INDIA

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