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Original Research Article

Biochemical and Microbial Characterization of Kurdi: A Traditional Fermented Food of Maharashtra

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A B S T R A C T

Fermented foods are one of the important items of the human diet. These traditional fermented food products are a household art prepared by using relatively simple procedures and equipments. Fermented foods like *idli*, *dosa*, *ambali*, *kanji*, *vadai*, *papad*, *kurdi*, jelabi, kharode, *bhaatijaanr*, *seera* etc, are some of the items largely consumed in Indian Subcontinent. Traditional fermented food products prepared in varied parts of India, have been well studied and recognized. However, there is no detailed study reported for foods like *kurdi an* indigenous food item prepared largely in state of Maharashtra, India. Hence, present work was undertaken to develop a scientifically sound, commercially viable and socially useful cereal based traditional fermented food products. Indigenous fermented product like *kurdi* was prepared from the naturally fermented flour/batter of wheat without addition of any chemical additives. Efforts were made to find out the effect of indigenous fermentation at varied time and temperatures on the biochemical characteristics and nutritional characterization with respect to pH, titratable acidity, starch, amylase and reducing sugars of *kurdi* from fermented flour/batter of wheat. Isolation and identification of micro flora responsible for fermentation of *kurdi* was carried out. Hence, this study provides an option to improve the process of making and more nutritious, a popularly consumed traditional fermented food, like *kurdi*. Since, it is a traditional fermented food; it is more nutritious and tasty. So, there is a thrust to introduce such easily adoptable, profitable and nutritious indigenous technologies as organic foods in the market.

Keywords

Kurdi, Starch, Amylose, Reducing sugars, Fermented batter of wheat

Introduction

The preparation of many indigenous or "traditional" fermented foods and beverages are remains today as a household art. In India, legumes alone or in combination with cereals, like rice constitute the basic ingredient of many fermented foods. In the passage of time, southern and western Indian fermented foods, like *idli*, *dosa* and *dhokla* have also become the choice for restaurant hunters as nutritious and delicious light midday meals.

Most of substrate changes while preparation of traditional fermented foods is due to enzymatic reactions. The microorganisms produce the proper enzyme which brings about specific transformations of substrate:

Improved flavour and appearance

Destruction of undesirable materials

Improved keeping quality

Enhanced nutritional value and

Improved digestibility, moreover, the product required less cooking time than the original substrate.

However, in some parts of world, huge amounts of fermented foods are produced and used in the daily diet of the people and are an essential part of their nutrition. Although these regions are quite far apart in geography, culture and religion, traditional fermented foods show some close similarities such as:

Most of traditional fermented foods are based on cereals such as millet, sorghum, maize and wheat.

The microorganisms used are typically those present in or on one of the ingredients and are selected by adjusting the fermentation conditions. Since almost all fermentations are self-inoculated.

Traditional fermented foods are essential for well-being of many people of the world, especially people of Southeast Asia i.e. India and South Africa (Hasseltine, 1979). Fermentation processes have been developed to upgrade plant and animal materials, to yield a more acceptable food, to add flavour to the blended cereal vegetable diet and to prevent spoilage and also to preserve food without refrigeration. These traditional processes are helpful to develop modern industrial enzyme fermentations and flavouring agents. Certainly the early food technologists knew nothing about fermentation process and their nutritional values. The diversity of the population of India has given rise to a large number of traditional fermented foods with cereals and legumes which have been extensively reviewed (Soni and Sandhu, 1990).

Wheat supplies about 20 percent of the food calories for the world's people and is a national staple in many countries. Industrial uses of wheat grain include starch for paste, alcohol, oil, and gluten. The importance of wheat is mainly derived from the fact that seed can be ground into flour. It also forms the basic ingredient of bread and other bakery products and presents the main source of nutrient to most of the population.

Kurdi is a traditional Indian cereal based fermented food prepared by soaking, fermenting and crushing wheat grains (Thakur *et al.*, 2004), which is subsequently thermally gelatinized, hand extruded and dried (Beuchat, 1983). The preparation of *kurdi* is an art of technology and is a family secret passed from mother to daughter. Since, time immemorial *kurdi* is known as a ceremonial fried food of special significance to the village people of Maharashtra. It marks a special occasion of the *Maharashtrians* such as marriage, religious and cultural festivals.

Similar type of food product named *Seera*, also called *Nishasta*, had been prepared in the state of Himachal Pradesh of India (Thakur *et al.*, 2004). Cereal legume based fermented foods like *idli*, *dosa*, *dhokla*, *khaman*, *wadi*, *papad* and *kinema* from various parts of India have been well studied and documented; however, there is no proper documentation of similar foods, indigenous to the state of Maharashtra (*kurdi*) in India (Nout and Sarkar, 1999; Nout *et al.*, 2007).

Traditional fermented foods are now being marketed as extruded foods or neutraceuticals. India has the specific advantage of varied cultures and such traditions are dying a slow death now in a fast-paced world**.** The purpose with the present work was therefore to elaborate a

scientifically sound, commercially viable and most useful wheat based fermented food product i.e. *kurdi*.

It has the advantage of being generally regarded as safe (GRAS) and offers immense opportunities for production of novel products which can be classified as a fermented foods, natural foods, convenience foods, health foods, functional foods, and neutraceuticals and not to forget foods for clinical nutrition.

Indigenous technology of food fermentation represents a distillation of knowledge and wisdom gained by trial and error basis. The knowledge of making traditional fermented foods has been recognized to be immense valve to the future generations. Fermented foods are becoming more popular worldwide because of their high nutritive value, organoleptic characteristics and easy digestibility.

Natural fermentation of cereals increases their relative nutritive value and available lysine. Starch and fibre tend to decrease during fermentation of cereals. Developments in various areas of food science have strengthened the belief of centuries and many traditional fermented foods are now being marketed as functional foods or neutraceuticals. India has the specific advantage of diverse cultures and therefore has a wide array of fermented foods that can be exploited for the benefit of masses.

Thus the present work was undertaken to develop a scientifically sound, commercially viable and socially useful wheat based fermented food product and the physicochemical and microbial characteristics of selected two cultivars of wheat used for preparation of traditional fermented food i.e. *kurdi* were studied.

Materials and Methods

Raw Materials

Wheat grains (*Triticum astivum* L., variety: PBN- 51 and PBN- 142) were procured from Wheat Research Station, Vasantrao Naik Marathwada Agricultural University, Parbhani, Maharashtra, India.

Chemicals

The culture media and chemicals used were obtained from Hi-Media laboratories Pvt. Ltd., Mumbai, India. Ethanol, Dinitrosalicylic acid reagent and potassium sodium tartarate (Rochelle salt) were procured from S.D. Fine Chem. Pvt. Ltd., Mumbai, India. Some chemicals obtained from Sigma Chemical Co. (St. Louis, MO) and chemicals and solvents used were of analytical grade.

Preparation of sample

The grains were sorted by removing broken grains and other unwanted materials. The wheat grains were soaked in water (1:3w/v) for a period of 15, 22.5, and 30 hr and were incubated at varied temperatures of 30, 37.5 and 45°C, for natural fermentation to take place. Water was replaced after every 6 hr so as to get fresh microbial growth and thus to have better fermentation of the wheat grains.

At the end of soaking period, the soaked water was discarded. The grains were rinsed twice with water and the softened wheat grains were ground in electrical grinder (Anjali make, Mumbai, India) at 2500 rpm for 30 s and kept at -20°C until analysis. Fermented wheat batter samples were dried in oven at 70^{o}C for 12 hr. The oven dried samples were ground in electrically operated grinder with stainless steel blades. The

ground samples were stored in polythene pouches with proper labelling and used for further investigation.

Proximate analysis

Determination of moisture

Moisture was determined by hot air oven method by drying the sample in hot air oven at 105 ^{o}C temperature until a constant weight (AOAC, 2010).

The moisture content of sample was estimated by the formula:

Moisture content (%) = Initial weight − Final weight /Initial weight ×100

Determination of ash content

Ash content of samples was determined using (AOAC, 2010) method. 5 g of sample was taken in porcelain dish dried previously, weighed and heated at 550±20 ^{0}C till constant weight was achieved.

Determination of crude protein

Crude protein (N×6.2) was estimated using the automatic KEL PLUS instruments, Pelican Equipments, Chennai, India by employing the standard methods of AOAC (2000).

Determination of fat

Fat was estimated using the automatic SOCS PLUS instrument, Pelican Equipments, Chennai, India by employing the standard methods of AOAC (2000).

Determination of carbohydrate content

Carbohydrate content was determined by difference.

Determination of crude fibre

Crude fibre was estimated using the automatic FIBRA PLUS instrument, Pelican Equipments, Chennai, India by employing the standard methods of AOAC (2000).

Thousand kernels Weight

Weight of 1000 kernels of each sample was determined.

Preparation of *Kurdi* from fermented wheat batter

Whole wheat bran (i.e. bran and germ portion) was separated from crushed softened (fermented) wheat batter of aforesaid cultivars by filtering through the muslin cloth. Filtrate was centrifuged (Remi Compufuge, Mumbai, India) at 3000 rpm for 10 min to settle down the batter. Supernatant was discarded and the remaining starch portion was cooked for 24 min by adding boiled water (1:1w/v) and salt (3%) with continuous stirring. The cooked batter was extruded using manual hand moulder in warm condition. Extruded samples (*kurdi*) were dried by placing on cloth in electrical cabinet drier at 45^{o}C for 8 hr and fried. The dried *kurdi* were packed in low density poly ethylene (LDPE) pouches and stored at ambient temperature.

Physico-chemical characteristics of fermented wheat

pH

The pH was measured by digital pH meter.

Titrable acidity

Titrable acidity was reported in terms of g lactic acid/100 ml using the standard method (Amerine *et al.*, 1967).

Moisture uptake

Procedure same as mentioned in *2.2.4.1*

Determination of starch

Starch from the sugar-free pellet was extracted in 52% perchloric acid at room temperature (Clegg, 1956) and was determined using anthrone reagent method.

Determination of amylose

Amylose was determined using the method outlined by Juliano (1971). 0.1 g fermented wheat flour sample was weighed accurately and taken into 100 ml Erlenmeyer flask, and 1 ml 95% ethanol and 9 ml 1 N NaOH were added. The samples were heated for 10 min in a boiling water bath to gelatinize the starch then were cooled and transferred into 100 ml volumetric flask and brought up to volume with distilled water. Five ml of the solution were pipetted into a 100 ml volumetric flask and 1 ml acetic acid (1N) and 2 ml iodine solution (0.2 g iodine and 2.0 g KI in 100 ml of aqueous solution) were added and the volume was made up to 100 ml and left for 20 min. The absorbance was measured at 620 nm by using spectrophotometer. Amylose content was determined by reference to a standard curve by using amylose standard. Amylose content was expressed as g/100g dwt.

Enumeration of total microbial/cell count

Total microbial counts in fermented mixtures were enumerated using nutrient agar, potato dextrose agar (PDA) and malt extract-glucose-yeast extract-peptone (MGYP) medium. Ten gram of unfermented flour of wheat sample was homogenized with 90 ml of 0.85% (w/v) sterile physiological saline and0.1ml of fermented batter of wheat sample was homogenized with 0.9 ml of 0.85% (w/v) sterile physiological saline in a Stomacher lab-blender (400, Seward, London, U.K.) for 1 min and serially diluted (10^{-7}) in the same diluents. One ml of these dilutions was prepared by pour-plate method in the respective media for bacteria, moulds and yeasts and incubated at 37^{o}C for 24-48 hr. The colony forming unit (cfu) were counted only after getting a final count in between 30-300 in nutrient agar plates using a colony counter (Nisha *et al.,* 2005).

Characterization of organism by Gram staining

Gram staining was performed to differentiate organisms, cells or its parts and to identify them partially. A smear was prepared and heated to fix. Primary stain (crystal violet) was flooded and allowed the slide for 2 min. Excess stain was drained off and washes with water by holding the slide in an inclined position. The smear flooded with Lugol's iodine solution and rinsed with water after 1 min. The slide was decolourised with 95% alcohol or acetone-alcohol (1:1 v/v) mixture, rinsed with water and dried. Counter stain the slide with saffranin for 2 min., washed, air dried and observed under oil immersion lens.

Growing, isolation and identification of microbial culture

The method used was based on those followed by Nout *et al.,* (1998); Banerjee and Sarkar (2003). One loop full of representative sample (0.1g/ml fermented wheat batter) was homogenized with 50 ml (each) sterile nutrient broth and potato dextrose broth using a Stomacher lab-blende. Incubated in an incubator shaker at 25 ^{0}C for 24-72 hr at 250 rpm or until the cloudy nature of broth occur, which seem to have vigorous growth of culture and then

O.D. was measured at 640 nm. Since, higher O.D. reading indicates the vigorous growth in PDB, hence the flask containing PDB was selected for isolation. The culture from PDB was isolated on PDA plates using spread plate techniques. Inoculate aforesaid samples on warmed petriplate containing 15-20 ml agar media and incubated at 37^{o}C for 24-48 hr. Single well grown colony was picked and transferred in Eppendorf having 1 ml of saline solution (0.9g of NaCl/100ml distilled water). Inoculated on newer plates and incubated at 37^{o}C for 24-48 hr for isolation. Repeat above procedure for 3-4 times until the isolated colonies were identical. Purity of the isolates was checked by streaking again on fresh agar plates of the isolation media, followed by microscopic examinations. Finally, isolated pure colony was transferred for identification/analysis of 18'sDNA and 26'sDNA.The identification was carried out at NCIM-CSIR, National Chemical Laboratory, Pune and results are reported. The identification report was generated using National Centre for Biological Information (NCBI/Gen Bank Database).

Statistical analysis

The results were statistically analyzed by one-way ANOVA using SPSS (IBM statistical analysis, Version 19). Duncan's new multiple-range test was used to determine significant differences. Statistical significance was declared at $p \leq 0.05$ post-hoc comparisons, SPSS 19 version (Duncan, 1955).

Results and Discussion

Physico-chemical characteristics of wheat samples

The untreated (control) wheat flour samples of PBN-51 and PBN-142 cultivars were analysed for physico-chemical composition and the results are presented in Table 1. The wheat flour contained 8.90% moisture,9.55 % protein, 2.70 fat, 1.48 % ash, 2.08 % fiber and 39.18 g thousand kernel weight respectively for PBN-51 cultivar, whereas, 8.32% moisture, 9.80 % protein, 2.72% fat, 1.49 % ash, 2.14% fiber and 31.51 g thousand kernel weight respectively for PBN-142 cultivar. The PBN-142 cultivar showed higher levels of chemical constituents than the PBN-51 cultivar. The moisture content of wheat flour was within the acceptable limit of not more than 10% for long term storage of flour (Singh *et al.*, 2005). The crude protein content, fat content and moisture content of wheat, differences can be attributed to be due to geographical location, type and varietal differences (Moss, 1987). The carbohydrate content of wheat flours was 74.22% reported by Ahmed and Lydia Campbell (2012). The findings of the study are in agreement with those of previous workers (Sharma and Khetarpaul, 1997).

Chemical characteristics of fermented wheat

The data on the effect of indigenous fermentation for a period of 15, 22.5, and 30 hr and fermentation temperature of 30.0, 37.5 and 45.0^{o}C in each of fermentation period on the chemical characteristics of PBN-51 and PBN-142 are presented in Table 2 and Table 3.

It was observed that at each fermentation period the protein content on dry weight basis was increased with increasing in fermentation temperature from 30.0 to 45.0^{0}C whereas, the fat content was decreased at all fermentation periods on increase in the temperature from 30.0 to 45.0^{0}C. The fat content was also significantly decreased on fermentation at a

temperature beyond 37.5^{0}C and 22.5 hr in PBN-51 cultivar; whereas non-significant reduction was found in both cultivars hence after 22.5 hr of fermentation period and 45^{0}C temperature. The same could be said for carbohydrate content which was decrease with increase in fermentation temperature and fermentation period. The fibre content was also significantly decreased on fermentation at a temperature beyond 37.5^{0}C and 22.5 hr of fermentation period for PBN-51 cultivar; whereas non-significant reduction was found hence after 15 hr. However, the significant reduction in ash content at 30^{0}C temperatures and 30 hr fermentation periods in PBN-51 variety was observed.

During the process of natural fermentation the carbohydrates (mainly starch) is degraded to ethyl alcohol and carbon dioxide due to natural synthesis of amylases. Therefore, the carbohydrates might have been decreased significantly while increase in protein content during fermentation could be probably because of hydrolysis of dry matter and synthesis of proteolytic enzymes which might have contributed to increase the total protein content at a slight rate. The increase in protein content can be attributed to microbial synthesis of proteins from metabolic intermediates during their growth cycles (Zamora and Fields, 1979). The protein content of sorghum is known to vary along with the changes in its amino acid composition (Waggle and Deyoe, 1966). Hadimani and Malleshi (1995) reported that ash content for pearl millet ranged from 1.2–2.4%.

Fat content was decreased after soaking and fermentation. Results are in agreement with El Maki *et al.,* (2007). Lipase enzymes hydrolyse fats into glycerine and fatty acids and since synthesized compounds are water soluble, they can diffuse into the cells tissue. The reduction in fat contents may be due to hydrolysis of dry matter and hydrolysis of fat occurred during fermentation.

pH and titratable acidity

Natural fermentation of wheat batter of both the cultivars was carried out at different temperatures (30, 37.05 and 45°C) for 15, 22.5 and 30 hr and results were reported in Table 4 and 5. It was observed that at each fermentation period the pH was significantly decreased with increase in water uptake and corresponding increase in titratable acidity with increasing fermentation temperature from 30.0 to 45.0^{0}C for PBN-51 cultivar; whereas, the pH was decreased at all fermentation periods on increase in the temperature from 30.0 to 45.0^{0}C for PBN-142 cultivar. After 15hr fermentation of wheat at 30, 37.5 and 45^{o}C, the pH declined with a corresponding increase in titratable acidity and water uptake (Table 4 and Table 5). The lower the temperature, the higher was the pH and the lower were the titratable acidity and water uptake; fermentation at 45°C gave the lowest pH and the highest titratable acidity and water uptake. A significant negative correlation was found between pH; water uptake and titratable acidity ($P \leq 0.05$).

Non-fermented wheat flour had initial pH 6.0 and titratable acidity 0.03g lactic acid/100 ml (Table 1) of PBN-51 cultivar. However, a significant decrease in pH (5.0) and corresponding increase in titratable acidity (0.18 lactic acid/100 ml) was observed when the wheat was soaked for 30 hr at 45^{0}C. A similar trend was also observed in pH and titratable acidity of PBN-142 cultivar formulated from soaked wheat (Table 5). However, decrease in pH and increase in titratable acidity was significantly higher in fermented wheat of cultivar PBN-51 and same trend was

observed in cultivar PBN-142. The increase in water uptake was more pronounced during 22.5 and 30 hr at 45°C in both the variety. The reduction in pH may be due to hydrolysis of starch into sugars during fermentation, which is readily utilised by the organisms and converted to lactic acid (Sripriya *et al.*, 1997). A rapid drop in pH with corresponding increase in titratable acidity has been reported in lactic acid fermentation of a number of foods including finger millet (Sripriya *et al.*, 1997) and cereal-legume blend (Sindhu and Khetarpaul, 2005). It was also reported that Lactobacillus spp. is more effective in lowering pH than yeast and a combination of microbes (Sangeeta and Khetarpaul, 2001).

Giese (1994) reported that, as a result of fermentation, the increased acidity and low pH enhances the keeping quality of cereals foods, by inhibiting microbial growth and also contributing to the flavour of processed foods. A rapid drop in pH with a corresponding increase in titratable acidity has been reported in lactic acid fermentation of various food grains (Lopez *et a1*., 1983; Nanson and Fields, 1984). The drop in pH is important in preventing the growth of food poisoning bacteria (Au and Fields, 1981). El Tinay *et al.*, (1979) reported that towards the end of the process, decrease of starch of fermented wheat was very small due to the drop in pH, which inhibited the activity of α and β amylases.

Starch, amylose and reducing sugars content of wheat

Table 6 and 7 show starch, amylose and reducing sugars content of wheat before and after fermentation of two cultivars. Unprocessed wheat ground flour contained 73.43% starch, 25.55 % amylose and 0.45 % reducing sugar respectively for PBN-51 cultivar whereas, 68.53 % starch, 24.27 % amylose and 0.42 % reducing sugar respectively for PBN-142 cultivar.

Significant ($P \leq 0.05$) decrease in starch content was observed at all fermentation periods with increase of temperature from 30 to 45^{o}C in both the cultivars. The decrease in starch content was from 76.42 to 64.15% for PBN-51 cultivar and from 72.16 to 63.17% for PBN-142 cultivar.

Amylose content ranged from 24.27 to 25.55% in unprocessed wheat. Amylose was higher in PBN-51 than PBN-142 variety. The amylose content was decreased significantly with increase of temperature from 30 to 45^{0}C at all fermentation periods for the both cultivars. Whereas, reducing sugar content was increased significantly at all fermentation periods with increase in the temperature from 30.0 to 45.0^{0}C. The reducing sugar content drastically increased by about 11-fold over unprocessed after 30 hr of fermentation, in both the cultivars. The PBN-51 cultivar was of higher starch content than the PBN-142 cultivar.

The decrease in starch content caused by fermentation could be attributed to yeast growth, breaking down sugars to ethanol and carbon dioxide (Pederson, 1971). An appreciable decrease in starch during fermentation may be attributed to hydrolysis of polysaccharides due to the action of α and β amylases produced by microorganisms (El Tinay *et al.*, 1979; Sindhu and Khetarpaul, 2005; Sripriya *et al.*, 1997). Considerable amounts of starch were hydrolysed at the beginning of the fermentation process. Moist heat may cause rupturing of starch granules followed by hydrolysis of starch to oligosaccharides and then to monosaccharides, which caused significant increase in concentration of sugars (Grewal and Jood, 2009).

Similar results were reported by Benmoussa *et al.,* (2006) and Dicko *et al.,* (2006a). The amylose content has been reported to vary with the botanical source of the starch, environmental conditions (Yano *et al.,* 1985) and genetic factors (Taylor *et al.,* 1997).

Microbial study

Wheat flour (ground) has a better carbohydrate profile for the growth and multiplication of micro flora, and it also lessens the chances of cooking losses of heat labile nutrients formed during fermentation of flour. Bacterial enzymatic hydrolysis has been shown to enhance the bioavailability of proteins by increasing the production of free amino acids. Lactic acid bacteria have also been shown to increase the content of the B-complex vitamins in fermented foods (Friend and Shahani, 1984). The fermenting microorganisms convert glucose to lactic acid, ethanol and CO_2. The drop in pH is important in preventing the growth of food poisoning bacteria (Au and Fields, 1981).

Enumeration of total microbial/cell count

Total microbial counts of non-fermented and fermented wheat flour/batter at 37 ^{0}C temperature for 15, 22.5 and 30 hr soaking period are presented in Table 8.

The results shows a significant increase in cell count ($P \leq 0.05$) was first observed after 15 hr fermentation period at 37^{o}C temperature and further significant increase in count at 22.5 hr and 30 hr of fermentation period of the two wheat cultivars. The growth of organisms in fermented wheat batter of var. PBN-51 and var.PBN-142 were found to be significantly higher (7.40 x 10^7cfu/mg and 7.50 x 10^7cfu/mg) as compared to the non-fermented wheat flour (3.28 x 10^4cfu/mg and 3.02 x 10^4cfu/mg). As the optimal temperature for the growth of organism was used, it appears that the cereal food supported the growth of organism well. However, increase in TPC for wheat grains soaked at temperature above 37.5°C (i.e. 45°C) was not appreciable. Thus, it appears that the growth of natural flora of micro-organisms was well supported when wheat grains were soaked at 37°C (Arora *et al.,* 2010). In the germinated food blend the increase in Lactobacilli count might be due to hydrolysis of germinated flours, which also provide better media for growth (Sripriya *et al.,* 1997). The slightly acidic and favourable environment increases the growth and activity of yeasts (Venkata Subbaiah *et al.,* 1985). TPC of the pearl millet increased with increase in soaking temperature; due to enhancement in the fermentation process (Sindhu and Khetarpaul, 2005). Microscopic observations shown that these isolated organisms from fermented wheat batter were in round or oval shaped, microaerophilic, gram-positive and ferment sugars to yield acids. Isolated organisms from 0.1g of fermented wheat batter showed better growth on PDA and violet purple colour colonies were seen in Gram stain. Strain was Gram positive and showed closest homology to *Clavispora sp.* using 26SrDNA and 18SrDNA sequencing.

Nutritional profile and frying characteristics of *Kurdi*

Moisture content of kurdi

The moisture content of *kurdi* had a positive impact on its texture and it mostly depends on the carbohydrate constituents of the wheat batter. The *kurdi* made in the traditional way (18 hr fermentation) from wheat batter of var. PBN-51 and var. PBN-142 exhibited a moisture content of 7.45% and 7.65% respectively.

It is clear from Table 9 that the moisture content was slightly less in *kurdi* as compared with unprocessed wheat. It was observed that moisture content of *kurdi* made from var. PBN-51 cultivar showed less value compared to that of *kurdi* prepared from wheat var. PBN -142 was found to be more fragile to handle. As per the Bureau of Indian Standards (BIS, 1972), moisture content in *kurdi* should be between 10.0 and 12.0%. Several other reports also indicate the variation in moisture content of *kurdi* prepared from different cereal blends (Shurpalekar *et al.,* 1970; Arya, 1992; Sangeetha, 1997). *Kurdi* become brittle and break if the moisture content is very low and prone for spoilage, if moisture is more than the desired level (Kulkarni *et al.,* 1996).

Nutritional profile of kurdi

Table 9 shows that the *kurdi* prepared from wheat var.PBN-51were found to have nutritional profile such as protein (10.13%), fat (1.72%), fiber (1.13%) and ash (12.04%). Whereas, nutritional profiles of *kurdi* prepared from wheat var.PBN-142 were protein (10.81%), fat (1.86 %), fiber (1.19%) and ash (12.06%). It was observed that nutritional profile of *kurdi* made from var. PBN-51 showed less values compared to that of *kurdi* prepared from wheat var. PBN-142. The results of this study indicated that soaking is one of the methods used to improve the nutritional value of wheat as raw material and it was also observed that protein and ash levels of *kurdi* increased after fermentation. The proximate analysis showed that *kurdi* made from fermented batter of PBN-142has higher nutritional quality attribute compare to *kurdi* of PBN-51. These constituents were slightly higher than those reported by Savitri *et al.,* (2000) and Gopalan *et al.,* (1996). These variations could be attributed to variety, season, species and stage of harvesting of wheat cultivar (Rangarajan and Kelly, 1996). The changes in chemical composition, amylose and minerals content after soaking and indigenous fermentation of two wheat varieties, named 'PBN-51' and 'PBN-142' were investigated. Protein content ranged from 9.55 to 9.80 % in unfermented wheat and PBN-142 was the highest variety in protein content (9.80%). PBN-142 was the highest variety in fat and ash content (2.72 and 1.49%).

Amylose content ranged from 24.27 to 25.55% in unfermented wheat and amylose was higher in PBN-51 was than other variety. Soaking for 30hr produced wheat with higher nutritional values having characteristics such as protein digestibility (84.95%), protein content (9.99%), fat content (2.64%), fiber (2.1%) and ash (1.37%). Little variations observed may be due to varietal difference and agro-ecological conditions of two cultivars of wheat.

Traditional methods of preparation of *kurdi* from fermented wheat batter thus, seem to have certain nutritional advantages. *Kurdi* offers unique dietary reward of not only improving the amino acid profile of wheat but also of making the starch and the resultant protein more digestible. Isolated *Clavispora sp.* organism from fermented wheat batter, may responsible for indigenious fermentation of wheat to make *kurdi*. Literature studies have reported possibilities of incorporating substances such as soy flour, cheese powder, cooked unripe banana and cooked colocasia into wheat batter and directly inoculated with aforesaid culture to make instant mix of *kurdi*. Acceptability of *kurdi* in terms of physicochemical and sensory characteristics and nutritional quality suggests the suitability of incorporating *kurdi* in a daily diet of human beings.

Hence, this study provides an option to improve the process of making and more nutritious, a popularly consumed traditional fermented food, *kurdi*.

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Table.1 Physico- chemical characteristics of wheat (control) samples*

Variety	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbo hydrate (%)	Fiber (%)	1000 Kernel Wt(gm)
PBN - 51	8.90± 0.03	9.55± 0.21	2.70±0.04	1.48± 0.01	77.25 ± 0.05	2.08 ± 0.06	39.18 ± 0.18
PBN-142	8.32± 0.14	9.80± 0.14	2.72±0.03	1.49± 0.01	76.77 ± 0.24	2.14 ± 0.08	31.51 ± 0.16

*Values are means ± (S.D.).

Table.2 Effect of indigenous fermentation on chemical characteristics of wheat var. PBN-51 on dry weight basis*

Fer. Period (hr)	Fer. Temp (°C)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)	Fiber (%)
15	30	9.61 a ± 0.01	2.69 e ± 0.01	1.48 e ± 0.01	77.23 g ± 0.01	2.02 f ± 0.01
	37.5	9.62ab± 0.01	2.68 e ± 0.02	1.47 de ± 0.01	77.21 f ± 0.01	2.00ef± 0.00
	45	9.64abc± 0.01	2.65 d ± 0.01	1.46 cd ± 0.01	77.19 e ± 0.01	1.98 e ± 0.01
22.5	30	9.65 bc ± 0.01	2.64cd± 0.00	1.45 bc ± 0.00	77.18 de ± 0.01	1.95 d ± 0.01
	37.5	9.67 cd ± 0.01	2.63bcd±0.01	1.45 bc ± 0.00	77.17 cd ± 0.01	1.93 d ± 0.01
	45	9.69 de ± 0.01	2.63bcd± 0.01	1.44 ab ± 0.00	77.16 c ± 0.01	1.90 c ± 0.00
30	30	9.72 e ± 0.01	2.62abc ± 0.00	1.44 ab ± 0.00	77.13 b ± 0.01	1.87 b ± 0.01
	37.5	9.85 f ± 0.01	2.61 ab ± 0.00	1.44 a ± 0.01	77.10 a ± 0.01	1.85ab± 0.01
	45	9.86 f ± 0.01	2.60 a ± 0.00	1.43 a ± 0.00	77.08 a ± 0.01	1.83 a ± 0.01

*Values are means ± (S.D.). Means not sharing a common letter in a column are significantly different at $P \leq 0.05$, as assessed by Duncan's multiple range test.

Table.3 Effect of indigenous fermentation on chemical characteristics of wheat var. PBN-142 on dry weight basis*

Fer. Period (hr)	Fer. Temp (°C)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate (%)	Fiber (%)
15	30	9.8 a ± 0.03	2.71 e ± 0.01	1.48 e ± 0.01	86.9 f ± 0.14	2.13 b ± 0.01
	37.5	9.82 ab ± 0.03	2.69 d ± 0.01	1.45 d ± 0.01	86.4 f ± 0.00	2.13 ab ± 0.02
	45	9.84 abc±0.01	2.69 cd±0.01	1.42 c ± 0.00	84.6 e ± 0.28	2.11 ab ± 0.01
22.5	30	9.85 bc ± 0.01	2.68bcd±0.01	1.41 c ± 0.01	84.1 e ± 0.14	2.11 ab ± 0.01
	37.5	9.87 cd ± 0.01	2.67 bc ± 0.01	1.39 b ± 0.01	83.9 de ± 0.14	2.1 ab ± 0.00
	45	9.9 de ± 0.01	2.66 ab ± 0.00	1.38 ab ± 0.01	83.2 cd ± 0.21	2.1 ab ± 0.02
30	30	9.92 e ± 0.01	2.66 ab ± 0.01	1.36 a ± 0.00	82.8 bc ± 0.14	2.11 ab ± 0.01
	37.5	9.97 f ± 0.01	2.66 ab ± 0.01	1.37 a ± 0.01	82.2 b ± 0.28	2.1 ab ± 0.01
	45	9.99 f ± 0.01	2.64 a ± 0.00	1.37 a ± 0.01	81.2 a ± 0.85	*2.1a± 0.00*

*Values are means ± (S.D.). Means not sharing a common letter in a column are significantly different at $P \leq 0.05$, as assessed by Duncan's multiple range test.

Table.4 Effect of indigenous fermentation on pH, titrable acidity and moisture uptake content of wheat var.PBN-51*

Soaking Temp. (°C)	Soaking Time (hr)	pH	Titrable Acidity (g / 100 g)	Moisture uptake (%)
Control	0	6.0 h ± 0.01	0.03 a ± 0.00	5.40 a ± 0.14
30	15	5.8 g ± 0.03	0.09 bc ± 0.01	18.40 b ± 0.28
	22.5	5.7 f ± 0.03	0.11 bc ± 0.02	32.70 e ± 0.14
	30	5.5 d ± 0.01	0.14 de ± 0.01	45.60 h ±0.28
37.5	15	5.6 e ± 0.03	0.08 b ± 0.01	26.20 c ± 0.14
	22.5	5.4 c ± 0.01	0.14 de ± 0.01	34.21 f ± 0.01
	30	5.2 b ± 0.01	0.18 f ± 0.01	46.00 i ± 0.14
45	15	5.4 c ± 0.03	0.12 cd ± 0.01	28.00 d ± 0.14
	22.5	5.2 b ± 0.01	0.17 ef ± 0.01	36.20 g ± 0.14
	30	5.0 a ± 0.01	0.18 f ± 0.01	46.05 i ± 0.07

*Values are means ± (S.D.). Means not sharing a common letter in a column are significantly different at P ≤ 0.05, as assessed by Duncan's multiple range test.

Table.5 Effect of indigenous fermentation on pH, titrable Acidity and moisture uptake content of wheat var.PBN-142*

Soaking Temp. (°C)	Soaking Time (h)	pH	Titrable Acidity (g / 100 g)	Moisture uptake (%)
Control	0	6.0 g ± 0.03	0.04 a ± 0.01	5.60 a ± 0.03
30	15	5.9 f ± 0.03	0.07 ab ± 0.01	20.3 b ± 0.03
	22.5	5.7 e ± 0.01	0.09 bc ± 0.01	34.01 e ± 0.01
	30	5.7 e ± 0.01	0.12 cd ± 0.01	45.20 h ± 0.03
37.5	15	5.6 d ± 0.01	0.08 b ± 0.01	22.40 c ± 0.03
	22.5	5.7 e ± 0.01	0.12 cd ± 0.01	38.30 f ± 0.01
	30	5.6 d ± 0.00	0.14 de ± 0.01	45.80 i ± 0.03
45	15	5.5 c ± 0.01	0.16 ef ± 0.01	24.01 d ± 0.01
	22.5	5.3 b ± 0.01	0.18 g ± 0.01	40.20 g ± 0.03

*Values are means ± (S.D.). Means not sharing a common letter in a column are significantly different at P ≤ 0.05, as assessed by Duncan's multiple range test.

Table.6 Effect of indigenous fermentation on starch, amylose and reducing sugar of wheat variety PBN – 51*

Fer.Period (hr)	Fer.Temp (^{0}C)	Starch (%)	Amylose (%)	Reducing Sugars (%)
0	Control	73.43 j ± 0.03	25.55 j ± 0.04	0.45 a ± 0.01
15	30	67.18 i ± 0.03	23.47 i ± 0.01	2.34 b ± 0.03
	37.5	67.02 h ± 0.03	23.25 h ± 0.02	2.78 c ± 0.01
	45	66.87 g ± 0.04	23.03 g ± 0.04	3.15 d ± 0.01
22.5	30	66.27 f ± 0.04	22.96 f ± 0.03	3.63 e ± 0.04
	37.5	65.88 e ± 0.04	22.79 e ± 0.01	3.95 f ± 0.01
	45	65.45 d ± 0.04	22.62 d ± 0.03	4.33 g ± 0.01
30	30	65.23 c ± 0.03	22.42 c ± 0.03	4.59 h ± 0.01
	37.5	64.88 b ± 0.03	22.22 b ± 0.03	4.84 i ± 0.02
	45	64.15 a ± 0.04	21.92 a ± 0.03	5.15 j ± 0.01

*Values are means ± (S.D.). Means not sharing a common letter in a column are significantly different at P ≤ 0.05, as assessed by Duncan's multiple range test.

Table.7 Effect of indigenous fermentation on starch, amylose and reducing sugar of wheat variety PBN – 142*

Fer. Period (hr)	Fer. Temp(^{0}C)	Starch (%)	Amylose (%)	Reducing Sugars (%)
0	Control	72.16 j ± 0.03	24.27 j ± 0.04	0.42 a ± 0.01
15	30	66.36 i ± 0.03	23.16 i ± 0.03	2.49 b ± 0.01
	37.5	66.02 h ± 0.03	23.05 h ± 0.04	2.88 c ± 0.03
	45	65.82 g ± 0.03	22.82 g ± 0.03	3.19 d ± 0.02
22.5	30	65.46 f ± 0.03	22.60 f ± 0.03	3.43 e ± 0.01
	37.5	65.17 e ± 0.02	22.36 e ± 0.03	3.67 f ± 0.04
	45	64.82 d ± 0.03	22.07 d ± 0.04	3.92 g ± 0.03
30	30	64.01 c ± 0.01	21.74 c ± 0.04	4.05 h ± 0.04
	37.5	63.56 b ± 0.03	21.15 b ± 0.04	4.44 i ± 0.01
	45	63.17 a ± 0.04	20.82 a ± 0.02	4.89 j ± 0.01

*Values are means ± (S.D.). Means not sharing a common letter in a column are significantly different at P ≤ 0.05, as assessed by Duncan's multiple range test.

Table.8 Microbial loads of non-fermented and fermented wheat flour/batter at different fermentation time (at 37 ^{0}C temperature)*

Fer. Period (hr)	Total Plate Count (CFU/mg)	
	Variety PBN - 51	Variety PBN - 142
0	3.28 a ± 0.03 x 10^4	3.20 a ± 0.03 x 10^4
15	5.90 b ± 0.06 x 10^6	6.20 b ± 0.03 x 10^6
22.5	7.10 c ± 0.03 x 10^6	7.20 c ± 0.06 x 10^6
30	7.40 d ± 0.03 x 10^6	7.50 d ± 0.06 x 10^6

*Values are means ± (S.D.). Means not sharing a common letter in a column are significantly different at P ≤ 0.05, as assessed by Duncan's multiple range test.

Table.9 Nutritional profile and frying characteristics of *Kurdi**

Parameter	PBN-51	PBN-142
Moisture (%)	7.45 ± 0.24	7.65 ± 0.28
Protein (%)	10.13 ± 0.21	10.81 ± 0.24
Fat (%)	1.72 ± 0.09	1.86 ± 0.07
Ash (%)	12.04 ± 0.12	12.06 ± 0.14
Crude Fiber (%)	1.13 ± 0.07	1.19 ± 0.09
Frying oil Temp ºC)	175.3 ± 1.1	175.8 ± 1.3
Oil uptake (%)	31.02 ± 0.46	29.09 ± 0.42
% expansion	31.46 ± 1.2	31.02 ± 1.4

*Values are means ± (S.D.).

Gram staining and Identification of Microbial culture

	Max score	Total score	Query Cover	E value	Iden	Accession
Clavispora lusitaniae strain NRRL Y-11827 26S ribosomal RNA gene, partial Sequence	1234	1234	100%	0.0	98%	JQ689030.1
Clavispora lusitaniae isolate AFTOL-ID 1318 28S large subunit ribosomal RNA gene, partial sequence	1230	1230	100%	0.0	98%	FJ176872.1
Clavispora lusitaniae strain WM 1036 28S ribosomal RNA (LSU) gene, partial Sequence	878	878	71%	0.0	98%	JN941196.1

	Max score	Total score	Query cover	E value	Iden	Accession
Clavispora lusitaniae strain LZ-5 26S ribosomal RNA gene, partial sequence	704	704	100%	0.0	100%	JQ686919.1
Clavispora lusitaniae strain ExoC21 26S ribosomal RNA gene, partial sequence >gb|GU454736.1| *Clavispora lusitaniae* strain ExoC11 26S ribosomal RNA gene, partial sequence	704	704	100%	0.0	100%	GQ396267.1
Clavispora lusitaniae strain ExoC55 26S ribosomal RNA gene, partial sequence	704	704	100%	0.0	100%	GQ396270.1
Clavispora lusitaniae strain ExoC36 26S ribosomal RNA gene, partial sequence	704	704	100%	0.0	100%	GQ396269.1
Clavispora lusitaniae strain ExoC26 26S ribosomal RNA gene, partial sequence	704	704	100%	0.0	100%	GQ396268.1

Fig.1 Culture isolated from fermented wheat batter

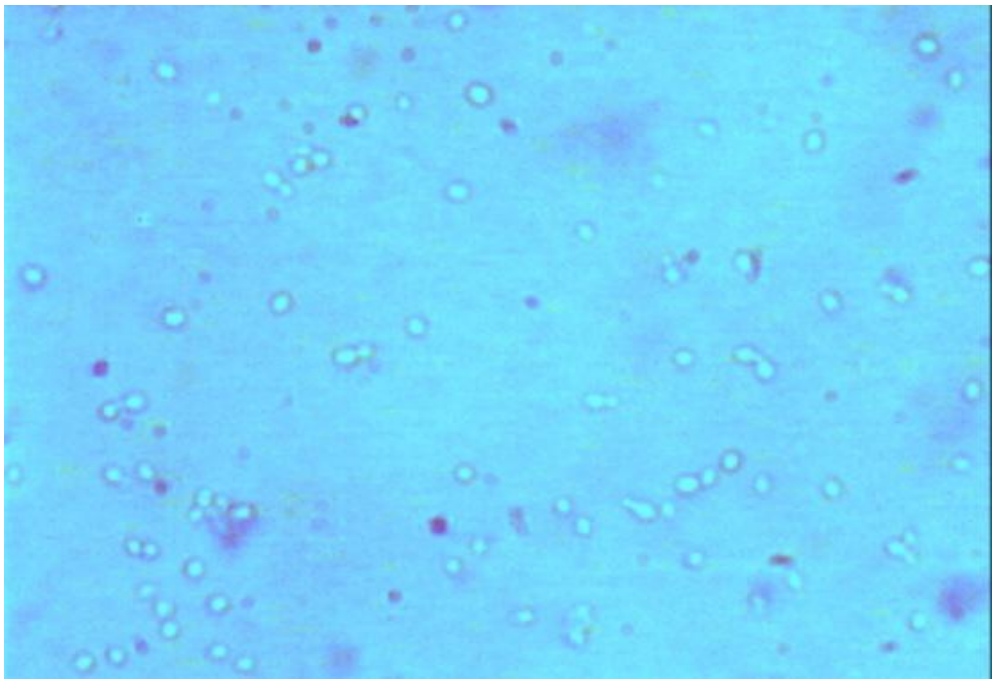

Fig.2 *Kurdi* (fried) prepared from fermented wheat batter var. PBN-142 and PBN-51

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