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RESEARCH ARTICLE

PHYSICO-CHEMICAL AND NUTRITIONAL EVALUATION OF WHEAT GERM *DOSA*:

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ABSTRACT

Popular staple fermented south Indian traditional food, *dosa* contains 30-40% expensive Black Gram (BG) contributing as protein source also as bacterial carrier, remaining is rice. Wheat Germ (WG), though a low-cost industrial by-product but filled with high quality protein (33%), PER-3, PDCAS 0.9, oil 14-16% (-3/6.2, -6/55.7), antioxidants like Tocopherols, Glutathione, Polyphenols, non-starch Polysaccharides and Minerals was studied as alternate to BG in the preparation of *Dosa*. Trials were carried out by replacing 25, 50, 75 and 100% of BG by Unflaked, Undamaged, Stabilized and Debitterized WG (USDWG) for *dosa* preparation. *Dosa* of 100 % USDWG had improved nutritional attributes, texture, and desirable sweet, sour, spongy texture with higher sensorial score and acceptability. USDWG incorporated batter had higher fermentation rate due to superior WG protein quality and quantity, enabling sought after fermentation time reduction to almost half, co-relating batter pH (4.4). Higher in vitro starch digestibility and viscoamylograph values confirm superiority of developed *dosa* which can emerge as ideal vehicle to alleviate malnutrition being low cost staple south Indian food. USDWG possesses strong anticancer (Dimethoxy Benzoquinone) and several disease preventive properties as observed by authors, thus, developed tasty *dosa* is therapeutic and health improving traditional food, at a very affordable cost.

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INTRODUCTION

Dosa and *Idli* are the popular staple traditional fermented food of south Indians prepared mainly from rice and black gram (BG). Rice is the source of carbohydrate whereas BG provides protein besides being carrier of much needed fermenting bacteria. *Dosa* is a fermented dish like *idli*, but the batter is thinner (lesser Consistency) more popular in the south Indian regions (Steinkraus 1996). The ratio of rice and BG in the mix, soaking time, grinding time, batter viscosity, particle size distribution of batter, fermentation time and preparation methodology of *Idli* and *dosa* differ significantly also *Dosa* is prepared by spreading fermented batter on heated oiled flat plate into a thin layer and cooked both sides to pleasant light brown colour. A sol to gel transformation occurs while cooking, within few minutes a circular, semi-soft to crisp product resembling a pan cake, ready for consumption (Battacharya and Bhat 1997). The biochemical changes occurring during natural fermentation include increase in non-protein nitrogen, total acids, soluble solids, methionine, cysteine and decrease in reducing sugars, pH and soluble nitrogen (Desikachar et al. 1960; Steinkraus et al. 1967).

During fermentation some microorganisms have been found to produce antimicrobial beneficial products that lead to safe and long storing of foods. Probiotics are live microbes associated with fermentation that, upon ingestion, beneficially affect their host by improving the balance of the intestinal microflora (Kalui et al., 2010) It is light on the stomach and is great for a good nutritional breakfast (Sulochana, Bakiyalakshmi 2011). Fermented legume and cereal products are becoming popular in the developed countries due to their nutritive value and organoleptic characteristics (Sanjeev and Dhanwant 1990). Recently, cost of BG, the main ingredient of *Dosa* steeply increased suddenly making it scarce for major section of Indian population, depriving them of good protein source and nutrition, exposing them to protein deficiency related diseases. An alternate affordable protein source was explored of similar functional, properties like BG, capable of producing products resembling *idli/dosa* of acceptable sensorial attributes. Attracted by the hidden nutritional virtues of WG, our team was successfully isolated/ extracted undamaged/unflaked, intact wholesome WG, first time, in a Commercial Roller Flour Mill. USDWG is excellent source of high quality protein 33% PER-3, PDCAAS 0.9, polyunsaturated fatty acids (-3, -6) vitamin E (richest vegetative source), mother of antioxidant Glutathione, poly-phenols, Vit - B and healthy fiber having strong anticancer properties.

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Attracted by the hidden nutritional virtues of WG, our team was successfully in-isolated/ extracted undamaged/unflaked, intact wholesome WG, first time, in a Commercial Roller Flour Mill. Nutritional and health beneficial properties of WG gets reduced to almost half if damaged or flaked during separation process. Though considered as health capsule, it is deprived of becoming part of regular human diet because it is highly unstable, turns rancid in no time and has slightly bitter, vegetative after taste. Therefore, our team after industrially isolating WG in its intact form, stabilized and debitterized it by combinatorial process of thermal and magnetic energy treatment (patent have been submitted at institute level). USDWG proved as the best affordable alternate to substitute BG in *dosa* preparation mainly with objectives (a) the Nutritional Gem, an industrial by-product which is essentially required by everyone every day (b) being cost affective (1/10 of BG), every segment of population can consume it thereby improve general health, alleviate malnutrition and prevent deadly diseases.

In a pilot clinical study it has been observed that Fermented Wheat Germ Extract (FWGE), when administered together with anti-inflammatory drugs in patients with severe rheumatoid arthritis (RA), could significantly improve disease outcome (Balint and others 2006). Fermented wheat germ extract prevents chemotherapy induced febrile neutropenia (Garami2004). The anticancer compound 2, 6-dimethoxy-1,4-benzoquinone (DMBQ) is the major bioactive compound in Fermented Wheat Germ Extract and is probably responsible for its anticancer activity (Christoph et al. 2016). The influence of lipids on lutein bioavailability in mice was in the order of Wheat Germ Oil (WGO)>Ground nut Oil (GNO)>control (mixed micelles). The relative bioavailability of lutein could be improved with WGO, and in turn may help to modulate the tissue fatty-acid profile and antioxidant molecules. This finding may imply a new insight for the dietary recommendations of lutein with WGO for improved lutein bioavailability (Gorusupudi et al., 2013). The present work has been carried out to study the Physico-Chemical properties of the batter and organoleptic evaluation of developed *Dosa*.

MATERIALS AND METHODS

Raw Materials: The raw materials selected for this study were (a) polished rice (*Oryza sativa*), (b) decorticated Black Gram (BG) (*Vignamungo*) were procured from local market, and (c) Wheat Germ (WG) was procured from M/s Basaveshwara Roller Flour Mill, Metagalli, Mysore, Karnataka employing the patented technology of authors.

Preparation of Dosa

The polished rice, decorticated BG and USDWG were soaked with potable water (Rice-150%, Black Gram-150% and USDWG-200%) separately for 4 hours. The excess residual water was drained, volume recorded and used later (to preserve and use soluble protein in the final product). Soaked rice, BG and WG ground in wet grinder (2 liter capacity) separately by adding required quantity of residual water (Grinding time for Rice-8 mins, Black Gram – 6 mins and USDWG- 6mins) batters were blended respectively in the proportion as shown in the Table No. 2. The batter was allowed to ferment for 14hours (overnight incubation at $32\pm 2^{\circ}\text{C}$).

Table1. Proximate composition of Rice, black Gram and Undamaged, Stabilized, Debitterized Wheat Germ (USDWG)

Parameters (%)	Rice*	Black gram*	USDWG
Moisture	13.3	10.9	13.0
Protein	6.4	24.0	32.0
Fat	0.4	1.4	16.0
Fiber	0.2	0.9	3.5
Minerals	0.7	3.2	5.0
Carbohydrates	79.0	59.6	35.0-45.0

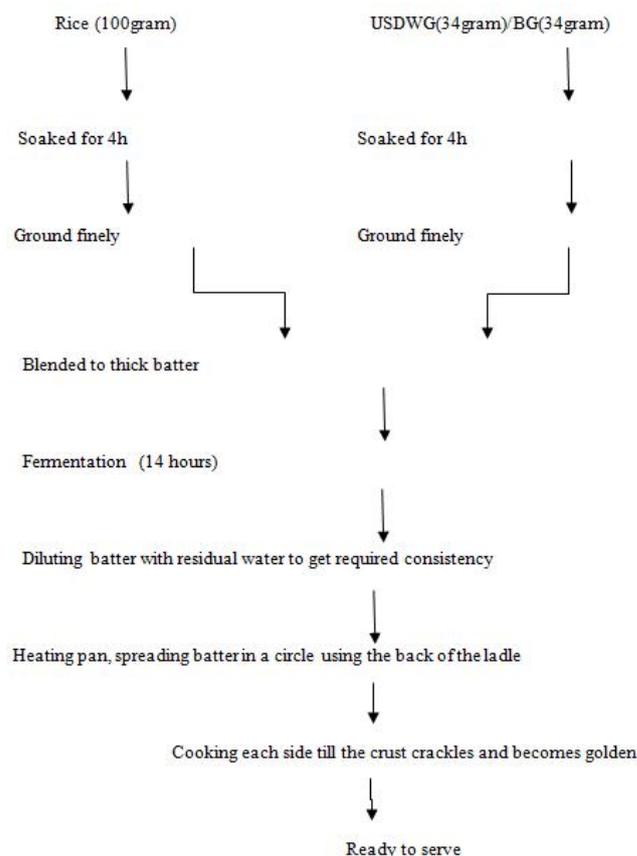
*Source: (Gopalan et al., 1971)

Table 2. Proportion of ingredients for dosa preparation

Samples	Rice (Grams)	Dhal (Grams)	Wheat Germ (Grams)
Control <i>dosa</i>	100	34	0
Wheat Germ <i>dosa</i>	100	0	34

The *dosa* was prepared in the study following exactly similar traditional preparation protocol for uniformity, easier adaptability and acceptance of the USDWG substituted *dosa*.

PREPARATION OF DOSA



pH and Density of the batter (g/cm^3)

Initial and final pH of the samples was measured using a pH meter (Cyberscan – Eutech Instruments, India). The batter density was determined by the volume of known weight batter before and after natural fermentation.

Measurement of CO_2

Amount of CO_2 released by the fermented batter was assessed using CO_2 Analyzer (phi dan sensor, Denmark) (Rekha et al., 2011).

Viscosity of batter

The viscosity of batter before and after fermentation (14 hours) was determined using Brookfield viscometer (Model DV-III, Stoughton, MA, USA) according to (Kim and Walker 1992), with slight modifications. *Dosa* batter was transferred to 100ml beaker and leveled up to the brim. The ASTM spindle speed was set to 100rpm at room temperature

Micro Viscoamylograph studies

The pasting properties of the flour/hydrocolloid blends were determined using the Brabender MVAG (Braebender, Duisburg, Germany). 10 grams of flour was weighed on a 14% moisture basis and shaken with 100 g water. The slurry was stirred in the MVAG at 250 rpm. The slurry was heated from 50 to 95°C at 6°C/min and then held at 95°C for 5 min. The slurry was then cooled back to 50°C at 6°C/min and held for 2 min. Peak viscosity, hot paste viscosity (HPV), end of cooling, final viscosity, breakdown, and setback values were all determined and reported in Brabender units (BU). The peak viscosity was the first maximum viscosity of the slurry. The HPV was the minimum viscosity during the hold period. The end of cooling was the viscosity of the slurry at the end of the cooling period. The final viscosity was the viscosity of the slurry at the end of the test. The breakdown is defined as the difference between peak viscosity and HPV. The setback is defined as the difference between the peak viscosity and the viscosity at the end of cooling (Ademolamonsur Hammed. 2016)

Nutritional attributes of developed DOSA

Proximate analysis

Proximate composition of the developed products viz, moisture, fat was analyzed according to the standard methods (AOAC 2005) and ash, protein (AACC 2000).

Color of the dosa

Color of the product was evaluated using Hunter lab color flex model DP – 9000 D25A in terms of Hunter L (lightness, ranging 0-100 indicating black to white), a (+a, redness and –a, greenness) and b (+b, yellowness and –b, blueness). View angle 10° (Nisha et al., 2005).

Texture analysis of 'dosa'

TPA (texture profile analysis) is a common test which uses the principles of compression. It's a kind of imitative test as it attempts to imitate the action of the jaw by compressing the sample piece twice in a reciprocating motion, also called as two bite test. This point indicates the beginning of the first compression and beginning of the second compression cycle. The force time curve obtained from this curve provides a number of textural parameters that are known to correlate well with sensory parameters. The TPA of the control and WG *Dosa* were conducted. Measurements were performed in three replicates and the average was reported in Newton's (force).

Minerals estimation

Mineral content of the samples was analyzed by using atomic absorption spectra according to the method described by (De-La Fuennea et al., 2003) with slight modification. Sample (5gm) was incinerated in a muffle furnace 570°C for 24 h. The ash obtained was dissolved in concentrated HNO₃ (2ml) and warmed for 5 min at 40°C in a water bath. The mixture was then filtered and analyzed by atomic absorption spectra (iCE 3000AA, Thermo Scientific, U.S.A).

Invitro Starch Digestibility (IVSD)

In vitro starch digestibility was analysed according to the method of (Goni et al., 1997) with some modification.

About 50mg of defatted sample was incubated with amyloglucosidase in acetate buffer (pH 4.6) at 60°C for 30min. This was centrifuged at 1500rpm for 15-20 min after inactivation of enzyme by boiling. The supernatant obtained was made up to 15 ml and 20µl was taken for analysis of glucose release using (glucose oxidase peroxidase) GOD-POD kit (span Diagnostics Ltd., Ahmedabad, India). GOD oxidizes glucose to gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase enzyme couples with phenol and 4-aminoantipyrine to form quinoneimine dye. The intensity of developed colour was measured at 505nm in spectrophotometer. Which is corresponding to hydrolysed starch and glucose released. Percentage of glucose release was converted to starch by multiplying glucose percentage with 0.9.

Sensory evaluation of the dosa

Dosa prepared with different batters were subjected to sensory evaluation by the method of Quantitative Descriptive Analysis (QDA) (Stone and Sidel 1998), employing qualified trained panel. During initial session, descriptors of the product were obtained by "Free choice profiling". Panelists were asked to describe the samples with as many spontaneous descriptive terms as they found applicable. The common descriptors chosen by more than one third of the panel was used in preparing a score card consisting of a 15 cm scale wherein 1.25 cm was anchored as low and 13.75 cm as high. The panelists were asked to quantify the perceived intensity of attributes by making a vertical line on the respective scale and writing the code number of the sample. They were also asked to indicate the overall quality of the product on an intensity scale which was anchored at very poor, fair and very good to assess the liking or preference of the product. The scores for all the attributes were tabulated and the mean values were calculated. 'Mean scores represented the panel' judgments about the sensory quality of the samples.

Statistical analysis

The experiments were conducted for three replications of each analysis. The collected data were compiled and analyzed by statistical methods using ANOVA test to evaluate the significant differences as described by (Steel and Torrie 1960).

RESULTS AND DISCUSSION

Changes in the pH during natural fermentation (14 hours)

Control and WG *dosa* batter had significantly different pH values; pH of the control batter at 0 hour was 5.94 which decreased to 4.5 at the end of fermentation period, whereas WG incorporated batter decreased from 6.1 to 3.5 respectively. It is evident from the pH values configured in Fig. 1. There is a higher acid production in the WG incorporated batter due to natural fermentation may be because WG is a good substrate for the microbial growth and presence of other required fermentation supporting nutrients consequently it shows higher fermentation rate compared to control batter. Enhanced gas production contributes to desired sour taste in the developed final product, also indicated in the sensory score card. This is mainly associated with the development of *S. faecalis* producing lactic acid which lowers the pH and production of carbon dioxide, which leavens the batter (Mukherjee et al., 1965). WG soaked in water has a high concentration of soluble nutrients to support the growth of lactic acid bacteria which can also be attributed to faster natural fermentation rate.

Table 3. Physical properties of the dosa

Samples	Texture		Color		
	Maximum load (N)	L	a*	b*	E
Control <i>dosa</i>	2.56±0.093 ^b	57.1±0.15 ^b	1.46±0.05 ^a	17.06±0.074 ^a	12.77
Wheat Germ <i>dosa</i>	2.45±0.08 ^a	48.4±0.016 ^a	2.4±0.033 ^b	18.34±0.033 ^b	21.60

Each value is mean of three replicates and followed by ±SD.

L- Lightness (black/white), a*- (green/red) and b*- (blue/yellow).

-Means in the same column followed by different letters differ significantly at (p 0.05). N-Newtons.

Table 4. Proximate composition of the dosa

Samples	Moisture	Ash	Fat	Protein	IVSD [†]
Control <i>dosa</i>	55.19±0.06 ^a	0.5±0.08 ^a	1.16±0.05 ^a	12.45±0.04 ^a	31.1±0.02 ^a
Wheat Germ <i>dosa</i>	58.63±0.03 ^b	0.7±0.053 ^b	3.2±0.04 ^b	15.36±0.021 ^b	36.3±0.01 ^b

Each value is mean of three replicates and followed by ±SD.

-Means in the same column followed by different letters differ significantly at (p 0.05).

IVSD[†]: Invitro starch digestability

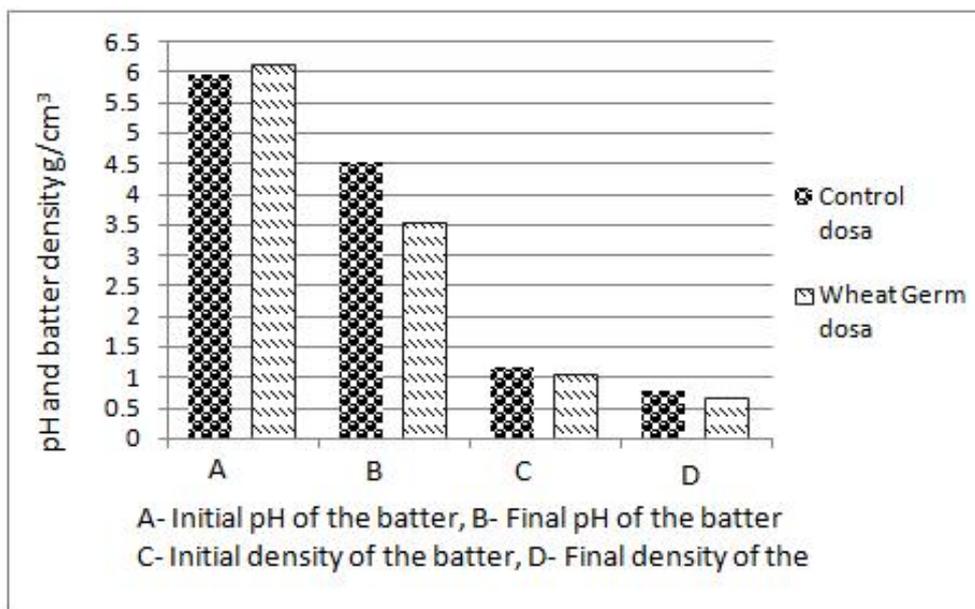


Figure 1. Changes in the pH and density of the batter during natural fermentation (14 hours)

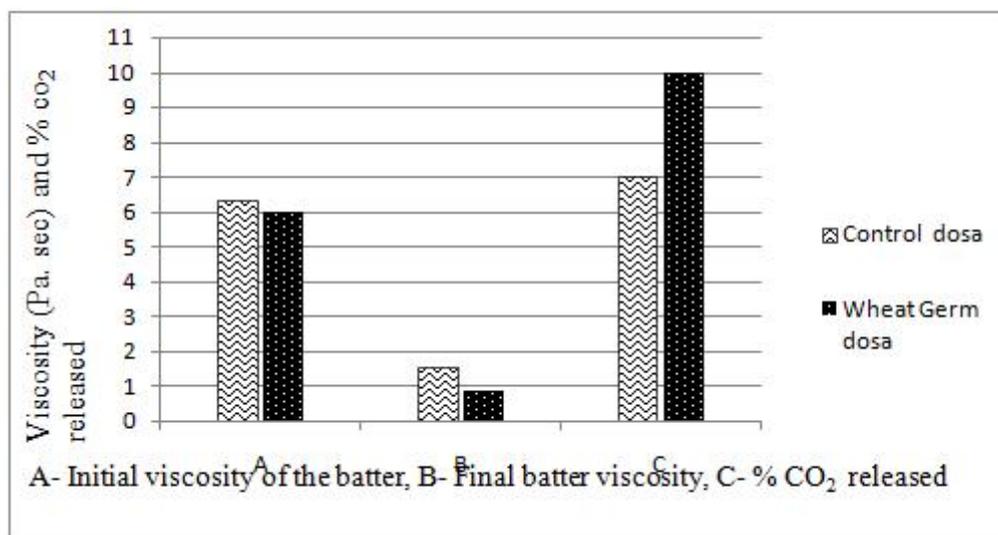


Figure 2. Changes in the viscosity and % of CO₂ released during natural fermentation (14 hours)

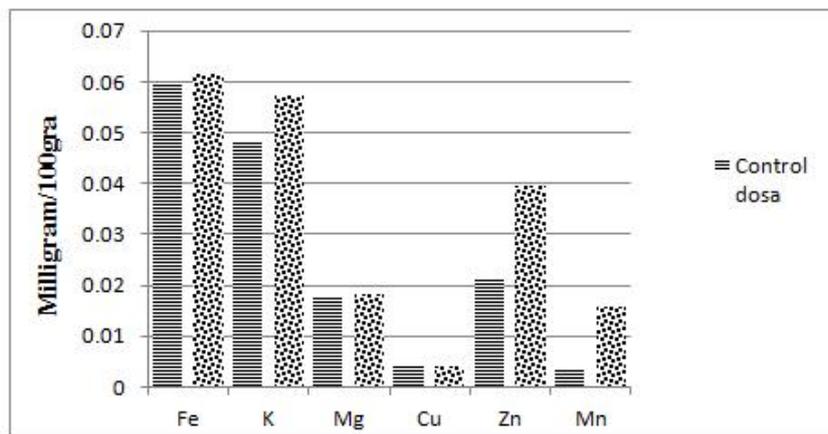


Figure 3. Estimation of Mineral

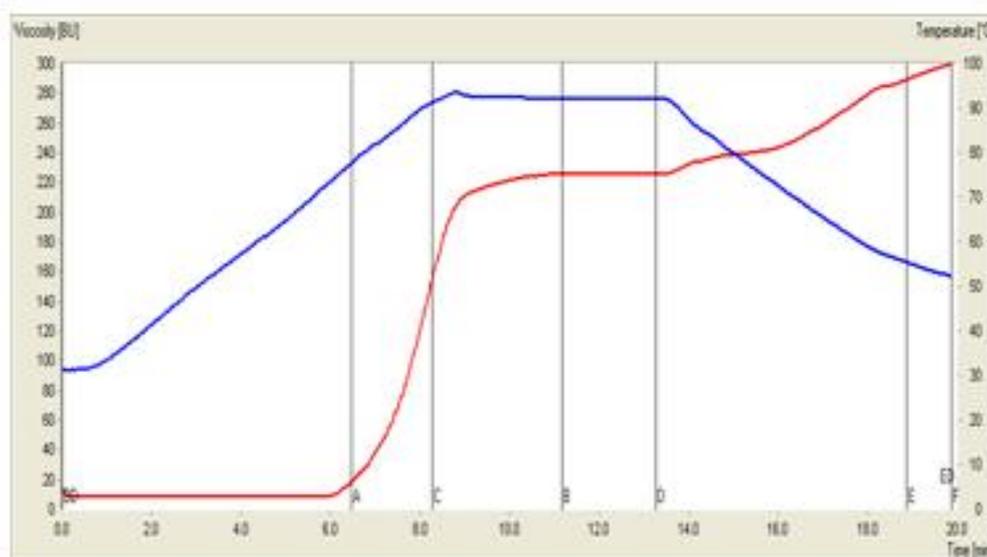


Fig 4(a). Viscoamylograph of Control dosa

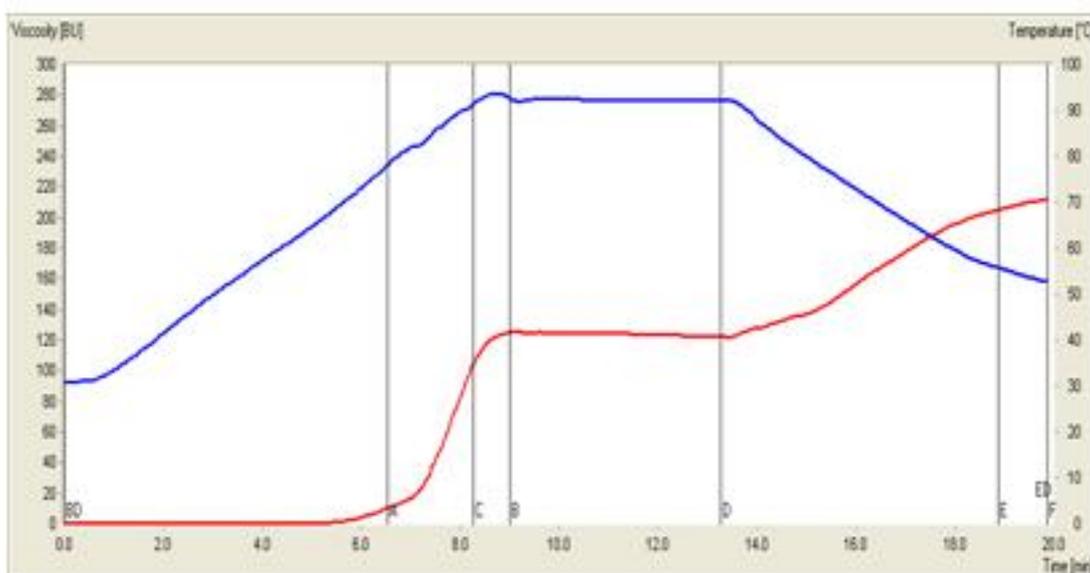


Fig 4(b). Viscoamylograph of Wheat Germ dosa

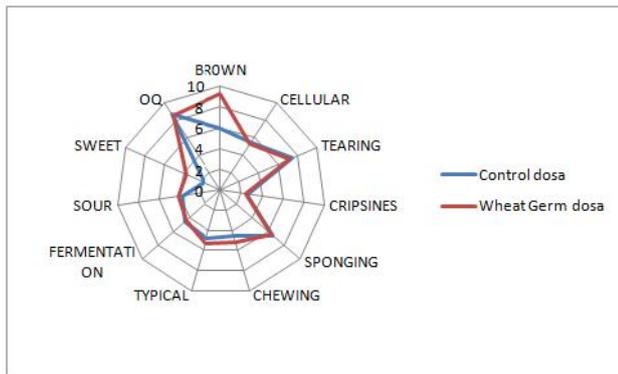


Figure 5. Quantitative Descriptive Analysis of the developed Dosa

The role of lactic acid bacteria is to reduce pH of the batter to an optimum level (4.5 to 3.5) for yeast activity (Soni and sadhu 1990). The final product gives good sourness and good sensory evaluation due to low pH value of WG incorporated batter.

Changes in the Density of the batter during natural fermentation (14 hours)

During natural fermentation, there was no significant difference in the initial density of control batter (1.13 g/cm^3) and WG incorporated batter (1.02 g/cm^3), but at the end of optimized fermentation period (14 hours) final batter density of control was higher (0.8 g/cm^3) whereas USDWG substituted batter was lower (0.64 g/cm^3) as shown in the Fig.1. Evidently, there is significant difference in the WG incorporated batter compared to control batter with regard to density variation during fermentation. Therefore, it can be conclusively stated, WG incorporated batter had higher rate of fermentation, higher volume, and good gas holding capacity which correlates with the softness of the final product. Density of the batter also depends on amount of water added to batter or amount of air incorporation. Increase in volume of *dosa* batter is due to the incorporation of lactic acid bacteria into the batter during fermentation and entrapment of air (Debasree Ghosh 2011).

Viscosity of the batter

Rheological study of the batters (Fig. 2) was carried out in terms of viscosity measurements using Brookfield viscometer. Control *dosa* batter recorded initial (6.3 Pa.s) and final (1.52 Pa.s) viscosity whereas WG *dosa* batter showed significant difference in initial (6.027 Pa.s) and final viscosity (0.86 Pa.s) which was considerably lower than control indicating higher rate of natural fermentation. Flow behavior indices were in the range of 0.86-1.52, which indicated strong non-Newtonian behavior (pseudo plastic or shear thinning) of *dosa* batter (Debasree Ghosh et al., 2011). It clearly reveals accelerated rate of fermentation that liquefied the WG batter. The finding of viscosity measurement confirms WG had increased rate of fermentation in agreement to our earlier statement.

Percentage of Carbon dioxide released during natural fermentation (14hours)

Rate of CO_2 gas production in the batter was assessed at different interval of fermentation period of control and combinations of WG blends.

The final stage of fermentation period after the elapse of 14 hours, control *dosa* batter had only 7% of CO_2 released; whereas WG incorporated batter exhibited 10% of CO_2 release as shown in the Fig. 2. Increased CO_2 production in the WG incorporated batter is due to higher nutritional attributes of WG as explained above, which helped in higher gas and acid production compared to control batter. It is been reported earlier that yeast is responsible for more than 50% of the CO_2 and two fold increase in the volume of the batter (Venkatasubbaiah et al. 1984).

Color determination of Dosa

Color of the control and WG *dosa* was analyzed using Hunter lab color flex model. WG *dosa* had more redness (2.4) and yellowish tinge (18.3) than control *dosa* (1.4) and (17) respectively. This is due to presence of carotenoids (Precursor of Vitamin A) and other nutritive components like to copherols and WG oil containing -3, -6 fatty acids. WG *dosa* had less degree of whiteness (48.4) compared to control *dosa* (57.1) (Table 3), probably due to having lot of health beneficial compounds with wide range of biologically active nutritional components.

Micro-viscoamylograph

Viscogram and data obtained by micro-viscoamylograph suggests WG *dosa* had delayed lower Gelatinization Temperature (GT) 10 BU compared to control *dosa* 19 BU indicating presence of resistant starch. Lower Paste Viscosity (PV) and cold Paste Viscosity of WG *dosa* 125 BU and 212 compared to control *dosa* having 226 and 299 BU respectively indicating WG *dosa* would be softer due to lesser retrogradation of starch. Break down 3 and 1 whereas set back values of WG *dosa* and control *dosa* were 83 and 64 respectively indicating comparable softness characteristics of the starch. Hence, *dosa* which are consumed hot will not have overall difference in eating quality of both WG and control *dosa* (Fig no. 4(a) and 4(b))

Texture of dosa

Textural profile of prepared *dosa* was carried out using texture analyzer and results were tabulated in the Table 3. Both control and WG *dosa* had similar shear value indicating similar and comparable textural properties. Maximum load offered by control *dosa* was (2.56 N) whereas WG *dosa* had (2.45N) suggesting WG *dosa* was slightly softer than control *dosa*. Softer texture of WG *dosa* was, maybe due to appropriate, adequate fermentation, higher gas production and leavening by stable gas entrapped bubbles due to better gas holding ability of the batter during natural fermentation. Increase in CO_2 production results in softness of the *Dosa* (Rekha et al. 2011).

In-vitro starch digestibility

Data obtained by estimating in-vitro starch digestibility of control and WG *dosa* were 31.1 % and 36.3% which clearly indicates better and easy digestibility of WG *dosa* Therefore WG *dosa* would be desirable for patients and people who need faster and higher calories. Table no.4

Minerals estimation

The content of Mg and Cu did not vary from control to wheat germ *dosa* (0.018mg and 0.004mg respectively). While the respective levels of Fe, K, Zn and Mg increased from 0.059mg

to 0.0614mg, 0.048mg to 0.057mg, 0.021mg to 0.039mg and 0.0035mg to 0.015mg compared to Control *dosa* (mg/100g). As shown in the Fig. 3

Sensory score

Sensory evaluation scores of control and WG *dosa* are showed in Fig. 5. Natural Yeast involved in the fermentation not only contributes towards gas production, which results in good texture also towards the sensory attributes of the fermented product like *idli* (Soni and Arora 2000). The difference in sensory quality of control and WG *dosa* samples were significantly distinguishable in some of the attributes viz sponginess, stickiness, beany, salty, sour taste, chewing, and overall qualities. WG *dosa* had more desirable sour and sweet taste along with chewy texture when compared to control *dosa*. This is due to more yeast growth in WG batter leading to higher rate of fermentation resulting desirable sour taste in the final product and due to the presence of higher pentose sugars in the WG. Overall quality score of WG *dosa* was 8.5 compared to control was 8.6 respectively signifying virtually similar in taste and other sensorial attributes.

Proximate analysis of dosa

The proximate analysis was carried out of the developed products to evaluate its nutritional level. WG *dosa* had higher moisture, ash, fat, and protein content 58.6%, 0.9%, 3.2% and 15.36% compared to control *dosa* 55.1%, 0.5%, 1.1% and 12.45% respectively as shown in the Table 4. It confirms WG incorporated *dosa* had the higher moisture, ash, fat, and Protein content than control *dosa* endorsing nutritional superiority of WG *dosa*. Accordingly, incorporation of WG in the batter exhibited higher nutritive values compared to BG. Therefore, it is advantageous in terms of nutritional and economic significance, to completely replace the BG with WG, to prepare traditional fermented food like *dosa*.

Conclusion

Successful attempt was made to develop traditional fermented food, *dosa* using WG, by completely replacing BG. WG is packed with nutrients especially high amount of simple functional proteins, which supports yeast fermentation exhibiting accelerated fermentation rate. WG *dosa* was superior to control with regard to protein and fat content, texture and sensorial attributes besides being more affordable cost wise. Higher values of in-vitro starch digestibility and viscoamylograph results conclusively suggest quicker assimilable quality of WG *dosa* hence it is more easily digestible than control *dosa* which goes in favor of newly developed *dosa*. The process being identical to the traditionally followed *dosa* preparing protocol, its acceptability and adaptability will be easy if marketed in the form of instant ready mixes or ready to use batters. Present invention has successfully converted an industrial waste to nutritionally dense traditional popular food which has the very strong potential to be best vehicle to eliminate, control and eradicate malnutrition of our country.

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