FERMENTATION KINETICS STUDIES FOR OPTIMIZED BLEND RATIOS OF HORSE GRAM, BLACK SOYABEAN BASED WARI

Thesis

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By

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CERTIFICATE

This is to certify that the thesis entitled ***"FERMENTATION KINETICS STUDIES FOR OPTIMIZED BLEND RATIOS OF HORSE GRAM, BLACK SOYABEAN BASED WARI",*** *submitted in partial fulfillment of the requirements for the degree of* ***Master of Technology in Agricultural Engineering*** *with major in* ***Food Biotech Engineering,*** *of the College of Post-Graduate Studies, G. B. Pant University of Agriculture and Technology, Pantnagar, is a record of bonafide research carried out by* ***Ms. Ankita Roy, Id. No. 42670*** *under my supervision, and no part of the thesis has been submitted for any other degree or diploma.*

The assistance and help received during the course of this investigation and source of literature have been duly acknowledged.

Pantnagar
June, 2013

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Chairperson
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CERTIFICATE

We, the undersigned members of the Advisory Committee of **Ms. Ankita Roy, Id. No. 42670,** a candidate for the degree of **Master of Technology in Agricultural Engineering** with major in **Food Biotech Engineering,** agree that the thesis entitled **"FERMENTATION KINETICS STUDIES FOR OPTIMIZED BLEND RATIOS OF HORSE GRAM, BLACK SOYABEAN BASED WARI"**, may be submitted in partial fulfillment of the requirements for the degree.

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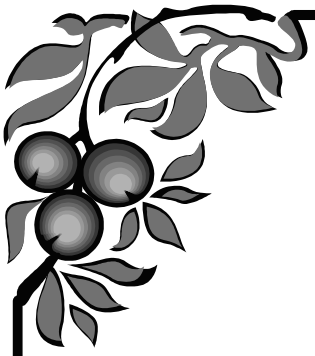
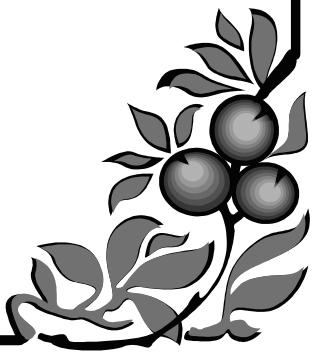
ANOVA	Analysis of Variance
Anon.	Anonymous
cm	centimeter
conc.	Concentration
db	Dry basis
DM	Dry Matter
dw	Dry weight
e.g.	for example
et al.	And others
etc.	Etcetera
F	F- Value
Fig.	Figure
gm	Gram(s)
gds	gram dry substrate
hr	hour(s)
ha	Hectare
HCl	Hydrochloric acid
i.e.	That is
kcal	kilocalorie
max	Maximum
M.C.	Moisture Content
mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeter
MT	million tones
NS	Non-Significant
OD	Optical Density
ppm	parts per million
qa	Quintal
R^2	Coefficient of Multiple Determinations

R	Coefficient of Regression
RSM	Response surface Methodology
rpm	revolutions per minute
SS	Sum of square
MS	Mean of Square
viz.	namely, in other words
WAI	Water Absorption Index
wb	wet basis
%	Per cent
/	Per
^{0}C	Degree Centigrade
µ	Microns
µ	Specific growth rate
µg	microgram(s)
U/g	Units per gram
v/v	volume per unit volume
s	second
w/v	weight per unit volume
w/w	weight by weight
Eqn.	Equation



Introduction



India has myriad sets of human population and there different variety of food habits has resulted in a large number of traditional Fermented Foods. With 40% share in total world production, India makes its place among the leading producers of legume and cereals**.**

The country possesses 7000 types of edible plants, but only few types of crops are widely used, out of which, maximum are underutilized crops. The production of these underutilized crops is around 821076 MT from approximately 522486 ha area reported during 2010-11 **(Directorate of Agriculture UK. 2011**).

In Uttarakhand state, some of the highly valuable and beneficial crops like black soya bean, horse gram, millets (finger millet, barnyard millet) are widely cultivated and consumed in the areas of lower Himalayan range.

Horse gram *(Macrotyloma uniflorum)* locally known as Kulthi is rich in iron, calcium molybdenum, polyphenols which have high anti-oxidant capacity and hemagluttinin which is a substance found in antibodies and autoimmune functions. In Uttarakhand, the area under this crop is about 11,032 ha with productivity of 6.19 qa/ha **(Directorate of Agriculture UK. 2011).** Horse gram is low in fat and is an excellent source of protein, dietary fibre, a variety of micronutrients and phytochemicals **(Kadam and Salunkhe, 1989; Siddhuraju and Becker, 2007).**

Use of horse gram flour, as ingredient in composite flours and functional foods, is limited, due to the presence of certain phytochemicals with antinutrient effects that limit the nutritive value of these legumes. Conventional processing methods, such as soaking, boiling, germination, and fermentation, are widely used to decrease the content of these undesirable components, which results in enhanced acceptability and nutritional quality in addition to optimal utilization of these legumes as human food **(Kadam and Salunkhe, 1989).**

Black soya bean is an important food crop of northern India especially Uttarakhand. Black soybean locally known as bhat/bhatmash, is grown in kumaon hill

regions in Uttarakhand, eastern Bengal, khasi hills and parts of central India **(Singh *et al*., 1970)**. It is a highly nutritious crop and good source of proteins, fibres and also it has better taste and nutritive value but due to presence of several anti-nutritional factor like trypsin inhibitor(TI), phytates, tannins, glycosides soybean process low protein digestibility **(Gupta, 1987)**

The consumption patterns of these crops are limited to traditional forms at local level only. One of the reasons for the unpopularity is their high anti-nutritional contents like tannins, phytate etc. But with the application of processing techniques, it has been found that level of these negative characters could be reduced to an acceptable level thus making the food product fit-to-consume.

One of such processing techniques is "Fermentation Technology". Though presently widely used at industrial level, the process finds its origin in ancient times at household level. Fermentation is a low-cost and the most economical technique of production and preservation of foods in developing countries. The technology not only reduces the anti-nutritional factor but can also enhance the nutritional content, digestibility, flavour and texture of food at a high level**.**

There are several other traditional pre-treatment techniques like soaking, malting, decortications and blanching which has been found to be beneficial in manufacturing of cereal-legume based value added foods by decreasing their anti nutritional content and increasing the nutritional level and digestibility.

Wari a legume based traditional fermented food is mostly consumed in northern Indian states. These are spicy brittle friable balls, cooked with vegetables. Wari is manufactured according to the traditional and less advance technology by using simple equipment and is produced on cottage and in household by women folk **(Pruthi *et al*., 1983),** using natural microflora from the staples and the surroundings. Since Wari is a dehydrated product, they have long shelf life and simple packaging and storage requirements.

Rural development in Uttarakhand is only possible when small scale food industries would be promoted. Since these industries involve lower capital investment

and rely on traditional food processing technologies, value added products based on underutilized crops of Uttarakhand could be prepared using fermentation technology and traditional methods. Traditional processing methods being adopted in the state are crude and not standardized. Also these methods are not based on sound scientific principles. Hence, upgradation of traditional processing methods is need to be done. Hence, keeping in view the above mentioned aspects of development, the present work has been planned with following specific objectives:

- Process standardization and optimization for development of wari using different blends of horse gram, black soybean, and black gram using fermentation technology.
- To see the effect of control (unfermented) and natural fermentation on wari quality.
- To study the fermentation kinetics and develop a model.

Review of Literature

Diversification of food production using underutilized crops must be encouraged both at national and household level in tandem with increasing yields.

Fermentation is a simple traditional household technology. Popularity of legume based fermented foods is due to desirable changes in legume that include texture and organoleptic characteristics, (aroma, flavour, appearance), elimination of beany flavors, improvement in digestibility (eliminating anti-nutritional factors) and increased nutrition. The organoleptic characteristics make fermented legumes more attractive to the consumer than the raw legumes.

Cereals- legume based Fermented food cover a wide range of food products such as papad, wari, idli, dhokla, dosa, jalebi, naan etc. Applying of fermentation process in utilization of horse gram and black soybean by product is a new concept. Proper utilization of underutilized crops can surely prove to be a boon for those low income groups who can't really afford to have the mainstream cereals.

Review work related to fermentation technology and value addition of underutilized crops has been compiled under following heading.

2.1 Fermentation Technology

Fermentation is one of the oldest and most effective methods of producing and preserving foods. Fermentation provides a natural way to reduce the volume of material to be transported, to destroy undesirable components, to enhance the nutritive value, appearance of the food and to reduce the energy required for cooking and to make a safer product.

2.1.1 Importance of fermentation

Fermentation of food products generally results in a value-added product having enriched nutrient compositions than the unfermented product.

Radhakrishnamurthy *et al.* (1961) reported that presoaking of the legumes releases the free sugars and non- protein nitrogen, which support the growth of lactic acid bacteria in traditional fermentation.

Mukharjee *et al.* (1965) reported that dehulled black gram harbors leconostoc mesenteroides and other lactic acid bacteria in large number, which play a major role in black gram fermentation.

Sudarlnadji and Markakis (1977) studied the changes in phytic acid during tempeh preparation by fermenting boiled soybeans with *Rhizopus oligosporus* . Boiling of soybeans resulted in reduction (14.0%) of phytic acid. About one third of the phytic acid was reduced in soybeans as a result of fermentation with mould *(R. oligosporus).* The decrease of phytic acid was accompanied by an increase in inorganic phosphorus. They concluded that t the reduction in phytic acid obtained, was due to the action of the enzyme, phytase, which was produced by mould during fermentation.

Reddy and Salunkhe (1980a) studied the effect of natural fermentation on phytic acid in black gram, rice, and black gram-rice blends. After 45hrs. of fermentation, about 13.3% of phytic acid was hydrolyzed in black gram.

Pruthi *et al.* (1983) reported that combination of pre-treatment and fermentation improved the nutrient quality of all underutilized crops and reduced the content of anti-nutritional factors to a safe level.

Akpapunam and Achinewhu (1985) studied the effects of fermentation on the chemical composition of Nigerian cowpea. Fermentation caused significant decrease in both phytic acid and phytate phosphorus. 34%, 61% and 69% reduction in phytic acid was observed after fermentation for 24, 48 and 72 hrs, respectively. There was no significant change in total phosphorus for beans fermented for 24 hours; after fermentation for 48 hours and 72 hours, significant increase in total phosphorus was observed. The decrease in phytic acid content was attributed to microbial degradation of phytic acid.

Boralkar and Reddy (1985) reported that progressive improvement in the protein digestibility with increasing periods of fermentation of soybean batter. Protein content was increased up to 29.41 % during 10 hrs of fermentation.

Sandhu and Soni (1989) studied the natural fermentation of black gram wari samples. The samples were drawn successively at 24 hrs interval for 5 days. Both

bacteria and yeasts increased significantly with the progress in fermentation. The increase in microbial load was followed by a decrease in pH and increase in dough volume The biochemical and physiological characterization of the predominant microorganisms indicated that both *leuconostoc* and *lactobacilli* perhaps produce acid and gas with the progress in fermentation causing acidification and leavening, leading to the fall in pH and rise in volume, thus making the environment unfit for other contaminations. The increase in total acidity as indicated by the fall in pH as a result of fermentation, probably helps in enhancing the shelf-life of wari and prevents the growth and transmission of various pathogenic microorganisms.

Marfo *et al.* (1990) reported substantial reduction in phytate levels of soybean and cowpea after seventy-two hours of fermentation. Lowering of phytate levels was most rapid within the first 48 hrs. of fermentation. The loss of phytate during fermentation was attributed to the activity of the enzyme phytase naturally present in the legumes. The drop in pH from 6.21 to 3.10, which was observed from dough during fermentation, probably contributed to the slow breakdown of phytate after 48 hrs. of fermentation.

Charl (1997) stated that a significant increase in the soluble fraction of food is observed during fermentation. The quantity as well as quality of the food proteins as expressed by biological value, and often the content of watersoluble vitamins is generally increased, while the antinutritional factors show a decline during fermentation.

Soni *et al., (*2001) reported the effect of temperature (28°C and 40°C) on wari fermentation (at 24 hr intervals, up to 5 days) of the sets of traditionally prepared dough, with initial pH between 5.5-6.0, by incubating the temperature in the temperature controlled rooms. They studies the effect of pH on wari fermentation also, by adjusting the initial pH of the dough to 4.5 and 7.5, respectively, with 1% citric acid and 1% sodium citrate and the effect of sucrose (1-2%) allowing the natural fermentation during drying of wari at room temperature. It has been observed that incubation at 28°C and 40°C favoured the rapid propagation of yeasts and becteria

respectively, which resulted in the product with rise in volume, decrease in pH, better appearance, taste, flavor and overall acceptability.

Inyang and Zukari (2008) reported that fermentation can be effectively used for improving nutritional quality of cereals grain by increasing protein content digestibility, available lysine content and relative nutritive value. Fermentation was also found to decrease trypsin inhibitory activity, amylase inhibitor activity, phytic acid and tannin contents of cereals.

Onweluzo and Nwabugwu (2009) reported that fermentation modifies some physical characteristics of cereals and legumes, increases the level of some nutrients, digestibility, bioavailability, and decreases level of anti-nutrients and increases nutrient density.

Chelule *et al.* (2010) reported that fermentation makes the food palatable by enhancing its aroma and flavour. These organoleptic properties make fermented food more popular than the unfermented one in terms of consumer acceptance. Fermentation modifies the food in diversified ways, resulting in new sensory properties in fermented product.

2.1.2 Effect of Fermentation on Nutritional Composition

Fermentation has proved to be an efficient and eminent bioconversion technique since time immemorial. Fermentation of food products generally results in a value-added product having enriched nutrient compositions than the unfermented product.

Hahasseltine (1979) observed that most of the cereal grains constitute a major source of dietary nutrients all over the world. Although cereals are deficient in some basic components (e.g. essential amino acids), fermentation may be the most simple and economical way of improving their nutritional value, sensory properties, and functional qualities.

Eka (1980) reported that phytate content in locust bean seeds was lowered from 0.51 mg/g to 0.31 mg/g by fermentation.

Reddy *et al.* (1982) stated that fermentation process is one of the oldest and most economical methods of producing and preserving food. The popularity of legume

based fermented foods is due to desirable changes in the legume that include texture and organoleptic characteristics. He also reported a reduction of 35.4% of phytic acid during idli fermentation.

Paredes-López and Harry (1988) stated that the quantity as well as quality of the food proteins as expressed by biological value, and often the content of water soluble vitamins was generally increased, while the antinutritional factors showed a decline during fermentation.

Kheterpaul and Chauhan (1990) stated that phytic acid could be reduced during fermentation of pearl millet in an increasing rate with increase in fermentation temperature.

Obizoba and Atii (1991) reported that combination of cooking and fermentation improved the nutrient quality of all tested sorghum seeds and reduced the content of antinutritional factors to a safe level in comparison with other methods of processing.

Steikraus *et al.* (1995) found an increase of 10.6 and 60% of methionine content in fermented samples over unfermented ones respectively, although they used different proportions of black gram and rice.

Liang *et al.* (2008) investigated the effects of soaking, germination and fermentation on brown rice with an aim to reduce the content of phytic acid, while maintaining sufficient levels of zinc, in the expectation of increasing its bioavailability. Fermentation treatments were most effective in decreasing phytic acid (56–96% removal), followed by soaking at 10° C after preheating (42–59%).

2.1.3 Fermented food product (wari) from Cereals/Legumes

Wari is a legume based traditional fermented food product, mostly consumed in Northern Indian states. These are spicy brittle friable balls, cooked with vegetables. Wari is manufactured according to the traditional methods and less advance technology by using simple equipment and is produced on cottage and household level by women folk.

Reddy *et al.* (1982) states that the popularity of legume based fermented foods is due to desirable changes in the legume that include texture and organoleptic characteristics (flavour, aroma and appearance or consistency), elimination of beany flavours, improvement in digestibility, enhancement of keeping quality of the product, absence of toxins and partial or complete elimination of anti nutritional factors, increased nutritional and reduced cooking time. The organoleptic characteristics make fermented legumes more attractive to the consumer than the raw beans or legumes.

Further, these foods can also help to eliminate nutritional deficiencies in the developing countries. Soybean, blackgram, moong beans bengal gram are the principal food legumes used in the preparation of legume based fermented foods. Food legumes can be fermented either alone or combination with cereals. Pulses-based fermented food comprise a diverse range of food products and most commonly consumed are papad, wari, dhokla, idli, vadai,dosa.

Sandhu and Soni (1989) reported the traditional method in practice for the preparation of Wari. Dehulled black gram was husked and soaked overnight in water and ground to a paste. The paste was added with asafoetida, caraway, cardamom, clove, fenugreek, ginger and red pepper and the resulting dough was moulded into small balls which assume the form of hollow, spicy and brittle friable balls 3-5 mm in diameter after undergoing simultaneous fermentation and drying in open air for 4-8 days.

Soni and Sandhu (1990 b) reported the wari making procedure, two sets of wari dough were prepared and fermented in laboratory employing the traditional procedure using dehulled black grams and the moong beans as the substrate. This involved the grinding of legume grains after overnight soacking and supplementing with several spices. The spiced paste was then molded into small balls and naturally fermented and dried in open air. The pattern of total microbial load and other biochemical and nutritional levels were studied in both the sets successively at 24 hr intervals for 5 days. Raw grains of moong beans used in the preparation of new doughs were also analyzed microbiologically and biochemically.

Kulkarni *et al.* (1997) followed the traditional method for manufacture of wari in Uttar Pradesh. After soaking, washing, again soaking and wet grinding of split

pulses, the paste was whisked well, until it became light and fluffy due to incorporation of air and it gave a shine to the dried product. The resultant paste was mixed with required quantity of spices. A general recipe for the preparation of wari included black gram split pulse (450gm), water (550), ashground shreds (100gm), black pepper whole (20gm), cumin seeds (20gm), red chilli powder (10gm), dried fenugreek leaves (100gm), dried coriander seeds (100gm) and a little quantity of asafetida, nutmeg and cinnamon. Wari was dried in sun and it was suggested that the product should be dried in mechanical cross-flow dehydrator, having perforated trays smeared with little quantity of edible oil, maintaining an initial temperature of 70°C after 2 hr, wari sample should be turned over in order to accelerate drying. The total drying time could be 8-10 hr in order to attain 8-9% moisture content in the product.

Neetu (2001) prepared an acceptable wari by using 10% grated and blanched mushroom during grinding to 90% black gram paste and spices, dry fenugreek leaves, coriander powder, and cumin seed powder and red chili powder of total dough. wari was dried in a tray dryer at a temperature of 70± 2°C for 8-10 hrs. She used Agaricus mushroom to improve quality of wari and their protein content. The size of wari was 10-15 gm each initially and after drying, stored in bags of 0.05 mm gauge.

Sharma *et al.* (2001) studied on the development of a gadget for palleting wari and reported the manufacturing process of wari. The splits of various pulses were soaked in water for 10-12 hr and ground by a mixer grinder. The ground material was kept for 36-48 hr for its proper fermentation.

2.2 Fermentation kinetics

Mukharjee *et al.* (1965) reported that the better volume generally reached a maximum within 24 after beginning of pre-soak of dhal or 16 hr following grinding and mixing of the dhal and rice (proportion 1:1).

Ramakrishnan (1979) studied the type of microflora in soya idli fermentation and has also characterized *leuconostoc mesentroides* to be the dominant organism responsible for lowering the pH of the batter.

Montville *et al.* (1987) studied the influence of pH on the type and concentration of metabolites produced from pyruvate by *Lactobacillus plantarum* in pH-controlled fermentors at pH values of 4.5 to 6.5. Specific growth rates, cell dry weights, and diacetyl concentrations were highest at pH 5.5, with values of 0.78 hrs^{-1}, 190 mg/liter, and 1.2 mM, respectively. While the conversion efficiency (millimoles of acetone formed per millimoles of pyruvate utilized) was highest (94.6%) at pH 4.5, acetone levels were similar (20 mM) between pH 4.5 and 5.5.

Antony *et al.* (2007) observed that the fermentation of finger millet resulted in decrease in pH after 6 hrs from 6.4 to 5.2 at 24 hrs and dropped further to 4.3 at 48 hrs. The titratable acidity increased steadily from 0.13 % to 0.7 % in 24 hrs and further rapidly to 2.5 % at 48 hrs. There was no further increase in titratable acidity or pH even up to 72 hrs was observed. The total microbial count increased exponentially up to 18 to 24 hrs and thereafter stabilized with a little decrease up to 48 hrs.

Omemu (2011) studied the fermentation dynamics during production of ogi, Nigerian fermented cereal porridge. The population of filamentous mould declined significantly during fermentation from 6.8 log_{10}cfu/g at 0 h to 3.7 log_{10}cfu/g at 12 hrs of steeping; thereafter no mould population was observed again throughout the fermentation period. The moulds isolated were *Aspergillusniger, Aspergillusflavus, Rhizopusnigricans, Fussariumsubglutinans and Penicilumcitrinum*. Continuous increase in yeast population was observed throughout the fermentation period. Similarly LAB population increased significantly from 4.65 log_{10} cfu/g at 0 h of soaking to 7.0 log_{10}cfu/g at 48 hrs soaking. The LAB isolates were identified as *Lactobacillus fermentum, Lactobacillus plantarum and Geotrichumfermentum*. The temperature of fermenting maize remained relatively constant between 28° to 30°C throughout the fermentation. The pH decreased and acidity increased during fermentation.

2.3 Wari processing

Pruthi *et al.* (1983) studied the optimum conditions for improvement in manufacture, packaging and storage of black gram and green gram wari. Technological

data on dehydration of urd and moong daal wari such as microbial load, tray load, number of wari per tray, drying ratio, mean weight of wari etc. had been reported. Potassium meta-bi- sulphite (KSM (0.1% level)) in each green and black gram wari proved to be the best antimicrobial agent for lowering the microbial load and for improving the color of the final product. Packaging and storage studies at a room temperature (13-42ºC) revealed that low density polyethylene bags of 300 and 400 gauge were quite suitable for packaging both types of wari for 10 months storage at room temperature.

Meena Tendon (1985) prepared traditional soyafortified fermented food wari by the mix of different blends ratios and evaluated protein content increase up to 22.47%. She also studied changes in anti-nutritional factors.

Tandan and singh (1987) added 10% defatted soy flour to green gram dhal batter. Wari prepared from this batter contain 9.2% moisture but the product was of very dark color and had poor rehydration characteristics.

Sandhu and Soni (1989) studied the microbiological analysis of all the fermented wari dough sample collected from the local market and those prepared in the laboratory. Studies revealed the occurrence of bacteria and yeast *Leuconostoc mesenteroides, lectobacillus fermentum* and *steptococcus faecalis* were the principle bacteria in wari dough fermentation causing acidification and leavening.

Kulkarni *et al. (*1997) revealed data for the physico- chemical attributes of black gram and green gram, procured from the manufacturing units. Technological data such as, moisture content, whisking time, drying ratio, time taken for sun drying, moisture content of dried product for preparation of different batches of black gram and green gram wari were determined and the range and mean values of each physic-chemical parameters (moisture content, protein, total ash, ether extractives, salt, acidity, ph, acid insoluble ash, carbohydrates) of black gram and green gram wari compared with the proposed standard values. Packaging and storage studies showed changes in moisture content and appearance of moulds or insects during storage at room temperature. Products had a shelf life of 6 months at room temperature in 120 gauge polypropylene bags.

Neetu (2001) prepared traditional fermented food wari by using 10% grated and blanched mushroom, standardized the process and evaluated the physic-chemical and sensory quality of mushroom fortified traditional food wari and studied the storage characteristics. She used *Agaricus mushroom* to improve quality of wari and their protein content. Mushroom wari (10% mushrooms) contain 27.3% protein, which was more than control sample having 20.2% protein.

Soni *et al.* (2001) reported an improvement in the nutritional and acceptable sensory characteristics of traditional panjabi wari, achieved through a combination of physico-chemical factors namely, incubation of wari dough with an initial pH of 4.5 and supplementation with 1-2% sucrose. Enrichment of dough with *sacchomyces cerevisiae* in combination with the native bacterial flora of the ingredients was found to be the best microbial environment for optimum wari fermentation.

Naveen kumar (2002) developed mushroom based wari and studied the storage characteristics. He used pleurotus species of mushroom 10% to 90% black gram paste. The initial moisture content of mushroom wari was 8-77% (db) and ERT was found to be 40.75% with water activity of 42%. The product was light brown in color, having hard texture and relative humidity of 52%. These products were found to be safe storage in bags with good shelf life.

2.3.1 Physico- chemical characteristics of Wari

Pruthi *et al.* (1983) studied the physico-chemical composition of black gram and green gram wari. The moisture content, crude protein, acidity, fibre, pH and carbohydrates were 6.2-13.6, 16-21.9, .8-1.8, 0.1, 5.1-5.7 and 5.1-5.7 percent respectively.

Meena Tandon (1985) studied the physico-chemical characteristics of soy-fortified wari. The moisture content, crude protein, fat, pH and carbohydrates were 7.29-3.96, 19.09-28.12, .0.93-1.02, 5.42-5.84 and 58.74-68.17 percent, respectively.

Neetu (2001) studied the physico-chemical characteristics of soy-fortified wari. The moisture content, protein, fat, ash, carbohydrates, pH and water absorption index were 8.7, 22.3, 1.0, 4.30, 63.7, 5.4 and 1.03 percent respectively.

Naveen Kumar (2002) studied the physico-chemical characteristics of soy-fortified wari. The moisture content, protein, fat, ash, carbohydrates and water absorption index were 8.8, 21.9, 0.97, 4.67, 63.64 and 1.42 percent respectively.

2.3.2 Rehydration characteristics of Wari

Meena Tandon (1985) studied the rehydration characteristics of soy-fortified wari. Hydration behavior of black gram wari fortified with various levels of soy-flour indicated that control black gram wari had the highest water absorption while those containing 30% defatted soy-flour had the lowest water absorption. There was gradual desrease in the water absorption of wari with the increase in the level of defatted soya-flour. The soy-fortyfied green gram wari had 140% water absorption after 4 hr of hydration against 238% in control. Between two types of product, soy-fortified black gram wari had somewhat lower water absorption than those of soy-fortified green gram wari.

Neetu (2001) reported that rehydration ratio of mushroom-fortified black gram Wari was found to be 3:08:1 against 2:50:1 of control sample.

2.4 Proximate composition and processing aspects of underutilized Crops

2.4.1 Proximate Composition of black soybean

Kapoor *et al.* (1975) studied the gross chemical composition content of 12 soybean varieties and reported that the values for crude protein varies in the range of 35.72 to 45.10 % and value of carbohydrates ranged from 14.64 to 30.29 %.

Sunderraj and Thulasidas (1976) estimated the proximate composition of black soybean and horse gram. They reported that black Soybean contains 36% protein and 18% oil and provide the most inexpensive sources of high quality protein and oil. Horse gram contains 18 to 29 % protein and also serves as a good source of minerals and other nutrients like crude fibre, carbohydrates and fat.

Odumodu (1992) chemically analyzed the soybean for oxalate and tannin. The varieties of soybean have the highest tannin content of about 0.15 mg/g. The presence

of these antinutrients makes plant (especially legumes) protein partially available with poor quality.

Hadimani and Malleshi (1993) reported bulk densities of soybean of different varieties i.e. dark brown, brown and creamish white as 0.59, 0.47 and 0.55ml/g, respectively.

Wolf and W.J. (2002) determined the nutritional components of black soybean. He observed 8.0% moisture content, protein content in range of 40 to 43.9%, carbohydrates content 30%, crude fat content 19-21%, crude fibre content 4% and 5.5% ash content in black soybean. He also reported the energy value of the whole raw black soybean around 400 Kcal per 100 g.

Egounlety and Aworh (2003) reported that the raw soybean has the highest amount of trypsin inhibitor (23.73 mg/100g) and can be effectively reduced up to 82.2% when cooked for 30 min.

Tarade *et al.* (2007) reported that trypsin inhibitor (TI) is also one of the antinutrients, which decreases nutritional value of a plant food by making plant proteins unavailable when consumed raw or semi-cooked.

Toledo *et al.* (2007) analyzed some of the antinutrients from five soybean cultivars and reported that the tannins content ranges from 0.01 to 0.39 mg/g. and trypsin inhibiter from 18.19 to 71.64 UTI/g.

Siddiq, M. *et al.* (2010) analyzed selected dry bean varieties (red kidney, small red kidney, cranberry and black) for the physicochemical properties. It was observed that the bulk density of the beans flour varied significantly from 0.515 g/ml for black bean flour to 0.556 g/ml for red kidney bean flour. Black soybean is considered as a source of complete protein, i.e., protein that contains significant amount of all essential amino acids. Soybeans also have the highest protein yield per acre of all crops.

Xu and Chang (2011) reported that, the black soybeans exhibited a higher phytic acid content than yellow soybeans. Dark coloured legume has a higher level of phytic acid and tannin content compared with light coloured legume varieties. Reduction of phytic acid content was observed to be in the range of 9–17%, and 10–19% in cooked yellow soybeans and black soybeans, respectively.

2.4.2 Processing of black soybean

Tawali *et al.* (1998) reported that in legumes, antinutrients are found to be predominantly in bran or outer covering and also in the germ. The raw soybeans contain significant amount of anti-nutritional factors such as phytates and trypsin inhibitor (TI). These are removed or destroyed during soaking, cooking and fermentation of the soybeans

Egounlety and Aworh (2003) observed that cooking significantly reduced trypsin inhibitors of all legumes. Tannins located mainly in seed coat were removed as a result of dehulling. Raw soybean had the highest amount of trypsin inhibitor (23.73 mg/100g) and can be effectively reduced up to 82.2%, in soybean when cooked for 30, min. It was also observed that, the combined effect of soaking, dehulling, washing and cooking affected on level of oligosaccharide to a greater extent. About 50% of raffinose and more than 55–60% of sucrose and stachyose reduced during processing showing the importance of these treatments. These changes are beneficial especially in infant feeding based on cereal and legume-based foods.

Ghavidel and Prakash (2007) studied the nutritional and antinutritional properties of green gram, chickpea and lentil in control, germinated and dehulled conditions. They observed that the germination caused significant increase in protein, thiamine, iron and calcium bioavailability and starch and protein digestibility contents of all the legume samples. Further increase in mentioned parameters was observed after dehulling the germinated legumes. It was also observed that phytic acid and tannin were reduced by 18–21% and 20–38%, respectively, on germination and more reduction was observed in dehulled samples over germinated samples.

Fernandes *et al.* (2010) studied the effect of soaking of common beans in water or other solutions (e.g. sodium bicarbonate, sodium chloride, acetic acid) to reduce the antinutritional factors, as well as to increase nutrient availability. They observed reduced phytates, phytic acid, total phenolic compounds and tannins. The mineral content was also reduced, but the bioavailability of most studied minerals was found to be increased. Also the effect of discarding the soaking water before cooking was found to be advantageous. This procedure seems to reduce some carbohydrate

fractions oligosaccharides of beans that cause flatulence. Therefore it was suggested that beans should always be soaked and the soaking water should be discarded before cooking when preparing beans to improve their nutritional quality.

Xu *et al.* (2011) studied the effects of boiling and steaming processes on the phytochemicals of two types of soybeans (yellow and black). They observed that the all thermal-processing methods caused significant decrease in total phenolic content and phytic acid content in all bean types as compared to those of the raw beans. It was concluded that thermal processing found to be best method for reducing phytochemicals in case of beans.

2.4.3 Proximate composition of horse gram

Ray (1969) analysed the chemical composition of horse gram. The moisture, protein, fat, carbohydrates and crude fibre content were 10, 22, 1, 55 and 5 per cent, respectively. The ash content was 3 per cent, whereas calcium and phosphorus content was 0.21 and 0.34 per cent, respectively.

Salunkhe *et al.* (1982) reported 28.80 to 71.2% value of total saturated and unsaturated fatty acids, respectively in horse gram. Palmitic and lionoleic fatty acids are principle fatty acids in horse gram.

Borhade *et al.* (1984) horse gram contains significant amount of sodium, potassium, manganese, copper and zinc 11.5 to 37.3, 322 to 762, 1.5 to 1.57, 1.81 to 5.5 and 2.8 to 3.4 mg per 100 g of seeds respectively.

Kadam and Salunkhe (1989) compared soybean and horse gram seeds and reported that the horse gram contains a significantly lower amount of phytic acid than soybean. They also reported that the presence of trypsin inhibitors was comparatively higher in horse gram than other commonly consumed legumes like chickpea, red kidney gram.

Sudha *et al.* (1995) determined nutritional composition of horse gram. They observed that the moisture content of horse gram ranging from 10.2 to 13%, protein 17.9 to 25.3%, the total ash content 2.7 to 3.8%, crude fibre 4.4 to 5.7%. Also the iron

content in horse gram has been reported to be in the range of 6.77 to 11.9%, calcium content 105 to 354 mg/100 g and phosphorus content was found to be in the range of 310 to 390 mg/100g. The tannins content in whole horse gram seeds as reported by varied between 763.3 to 895.9 mg per 100 g, which decreased upon dehulling of the seeds (215.3 to 361.9 mg/100 g).

Bravo *et al.* (1999) studied some nutritional content of horse gram and reported that the total dietary fibre content was found to be 22.47% of dry matter and it composed mainly of insoluble dietary fibre (21.61% of dry matter) with soluble dietary fibre being only 0.86% of dry matter. The value of carbohydrates was found to be in the range of 57.2% to 60.9%.

Chopra and Sankhla (2004) reported that tannins, the complex mixture of phenolic compounds, present in the seed coat of the most of the legumes are effective inhibitory agent of iron absorption.

Gopalan *et al.* (2004) determined nutritional composition of horse gram. They observed that the moisture content of horse gram ranged from 10.2 to 13%, total ash content 2.7 to 3.8% and crude fibre 4.4 to 5.7%. The energy value of the whole raw horse gram was found to be 321 Kcal/100 g. The phytat phosphorus in whole horse gram was observed in ranged between 117 and 184 mg/100 g.

Nimkar *et al.* (2006) reported that at moisture content of 9% the average length, width and thickness of grains were 5.9, 4.0 and 1.9 mm respectively. The bulk density, true density and porosity decreased from 823 to 763 kg/m3, 1380 to 1250 kg/m3 and 40 to 38.9% respectively, as moisture content increased from 9% to 31%.

Sreerama *et al.* (2008) reported that utilization of horse gram and its flour in legumes composite flour and products is limited due to the presence of anti-nutritional components, poor functional and expansion properties. It is widely distributed in several legumes.

Zawadzki *et al.* (2010) reported that the tannin content in the horse gram seeds is different with varieties and it has been divided into low and high-tannin ones. In the low-tannin varieties, the content of tannins in the coated seeds ranged from 0.026 to

0.04 mg/g dry mass, and in case of higher it is ranged between 0.594 to 0.755 mg/g dry mass.

2.4.4 Processing of Horse gram

Puffing is a process that involves the release and expansion of a gas from the inside of a product to cause expansion or rupture of an existing structure in order to create an aerated, porous, snack-like texture with the added flavours.

Sudha *et al.* (1995) estimated proximate nutrients, calcium and some anti nutrients in 16 varieties of whole Horse gram and their dehulled seeds. The protein, fat and carbohydrate contents were higher in the dehulled samples than in the corresponding whole horse gram. However, the moisture, fibre and ash and calcium contents of the dehulled samples were lower. A significant portion of the anti-nutrients were removed by dehulling.

Zapotoczny *et al.* (2006) studied effect of temperature on physical, functional and mechanical characteristics namely seed size, shape, colour, water and fat absorption, resistance to compression and back extrusion energy of hot air puffed amaranth seeds for designing apparatus for puffing cereals with hot air. During the study, they observed that amaranth seeds processed at 290 ^{0}C are characterized by acceptable colour, physical, functional and mechanical properties.

Siddhuraju and Becker (2007) reported the effect of conventional processing methods on horse gram, such as soaking, decortications, popping, and fermentation, widely used to decrease the content of undesirable components, which results in enhanced acceptability and nutritional quality in addition to optimal utilization of these legumes as human food.

Sreerama *et al.* (2008) studied the nutritional implications and flour functionality of popped Horse Gram and gave the proximate composition of dehusked raw dhal and expanded dhal. The protein content of Horse Gram dhal was comparable to other legumes however xylanase treated expanded dhal had higher protein compared to dehusked raw dhal and untreated expanded dhal differing statistically ($p < 0.05$). Degradation of some of the cell wall non starchy polysaccharides by xylanase resulted

in a lower carbohydrate and a higher protein content in the enzyme treated grain. Raw and processed Horse Gram contained similar content of the total dietary fiber in the range of 14.57-16.14%.

Sreerama *et al.* (2008) reported that utilization of horse gram and its flour in legumes composite flour and products is limited due to the presence of anti-nutritional components, poor functional and expansion properties. They observed that the popping/expansion of horse gram improved the functional, expansion properties significantly by reducing the level of anti-nutritional components like phytic acid, tannins and trypsin inhibitor up to 60%. Horse gram were soaked in three volume (w/v) distilled water for 4-5 hrs to attain saturation. The grains at saturated moisture content (57%) were popped using hot sand (1:6) at temperature ranging from 230 to 250^{0}C for a short time (20-30 sec). These high temperature short time (HTST) conditions used during expansion process lowered the levels of phytic acid, tannins and protease inhibitors by 46%, 61% and 92%, respectively.

Conclusion

From the presented reviews, it can be concluded that, the underutilized crops offer wide range of opportunities for utilization in diversified products along with better nutritional qualities. Also role of fermentation technology has been discussed. But still very less literally material has been found on the standardized conditions for the Wari production. Research work related to optimization of processing conditions and standardization of sprocess for making Wari using fermentation technology needs to be done.

Materials and Methods

Experiments were conducted to standardize the process for formulation of wari using Horse gram, Black gram and Black soybean. Blend ratios were optimized and effect of blend ratios on quality attributes was determined. Product was formulated using fermentation technology. Fermentation kinetics was also studied for the optimized blend ratio.

The experiments were conducted in two phases. In the first phase, standardization of process for horse gram, black soybean and black gram based wari was developed. Three levels (50g, 100g, 150 g) of each grain horse gram, black gram and black soybean were kept for the study respectively. All the samples were divided into two lots. One lot of each sample was kept for natural fermentation and the second lot was kept as a control sample (without fermentation).

Constant parameters i.e. water and raw material ratio, mixing time, fermentation period, soaking period, drying time, sample size, drying temperature, were kept on the basis of preliminary trails result and review basis. Dependent parameters (nutritional, anti-nutritional, functional), sensory evaluation and rehydration ratio were determine to check the quality of the end product.

In the second phase of the experiment fermentation kinetics for the optimized blend sample was conducted and model were developed. The data analysis was conducted using the software design expert.

The details of the materials and equipment used, the design of the experiments, the experimental procedure followed and data analysis are reported below.

3.1 Materials and Equipment

3.1.1 Raw Materials

The raw grains i.e. Black soybean (*Glycine Max)*, black gram and horse gram (*Macrotyloma uniforum*) of traditional varieties and cucumber required for the study

were purchased from the local market of Haldwani and Bheemtal. Grains were cleaned, dried and kept in moisture proof plastic containers for further study.

3.1.2 Equipment

The list is enclosed as Table 3.1

Table 3.1: List of equipment/instruments used and their specifications

Equipments/ Instruments	Make and Specifications	Purpose
Autoclave	Yorko make, Vertical,	Sterilization of medium, glasswares
Centrifuge	Sigma laboratory Centrifuge,0–6000 rpm,	
Mixer Grinder	Picasso mixer grinder, 600 watt, 18000 rpm.	To obtain solid mass separated from liquid
Tray Drier	MAC Tray Drying Oven, CAT.NO.: MSW-216	To grind the samples
		To dry the samples
Electronic Balance	Mettler AE 166, Capacity 100 g, Least count: 0.0001	To weigh the chemicals
Spectrophotometer	Beckman, Model DU -7, Light source: UV – VIS, 220 V, single beam	To measure the light absorption or transmission

3.2 Experimental methodology

The procedure for the preparation of wari is given in the Flow diagram in (Fig. 1).

In the present study, first the raw grains (horse gram, black gram and black soybean) were evaluated for their proximate compositions (protein, tannin, phytic acid, and water absorption index). For the first lot (Natural fermentation) grains were soaked separately in to tap water (2:1 w/v) for 8 hrs. After 8 hrs the water was drained off. Dehusking was done for soaked grains manually (using muslin cloth) separately. Then the grains were washed repeatedly with adequate quantity of tap water. Water was

drained and blotting paper was used to absorb the moisture. Grains were then wet grinded separately in a mixer grinder with 4% of water (grinded up to 3 min to get a smooth paste). The 17 different blend ratios were prepared taking, 3 levels for each grains (50g, 100g, 150 g). After preparing the blends (as per design), finally 200g from each run was taken out for conduct the experiment. The batter was divided into two lots. First lot was used subjected to natural fermentation and the second one was kept on it is (control).

The period o natural fermentation was kept as 12 hr at room temperature. Slice cucumber peeled was added into the both lots. For this cucumber was sliced, peeled and seeds and inner spongy parts scrapped off Slices were grinded in the grinder and then squeezed in a muslin cloth to remove the water from the mash. The squeezed mash was added (10% of the batter volume) to non-fermented batter and (10% of the batter volume) to fermented batter. Small quantity (1% of batter volume) of water mix of cucumber was then mixed with the batter. The mixture was manually whipped for 10-15 min for uniform mixing of cucumber paste and batter. Then the wari batter was divided manually into balls of about 10 gm each and spread on trays in Tray drier. Wari was then dried in a Tray dryer for 7-8 hr at 70±1ºc temperature upto the mc comes to 8-9% of the final product. The drying time to obtain the final product for each sample was determined on the basis of preliminary trails. Nutritional (protein), anti-nutrition (phytic acid, tannin) and functional properties (water absorption index), sensory analysis and rehydration ratio of all blend ratios were determined. Optimized the blend ratios using RSM box benkhen technique was done on the basis of dependent parameters.

In the second phase, optimized blend ratio (from the Phase 1) was used for the fermentation kinetic study. A 100 g batter of optimized blend ratio sample was taken in a 250ml measuring cylinder and kept for fermentation at room temperature (28-30ºC). a graph strip was pasted on the measuring cylinder to enhance its volume measuring accuracy to about 1 ml.. The volumes of the fermented batter were noted periodically. The volume vs. time plots were developed and the time for maximum volume, i.e.

maximum fermentation was estimated. The volume vs. time data at fermentation at room temperature was used for kinetic model development.

3.3 Experimental Design

The Final experiments were designed using Box Benkhen design (as shown in Table 3.6). Following constant, independent and dependent parameters (as reported in Table 3.2, 3.3 and 3.4) were taken into consideration.

Table 3.2: Constant parameters

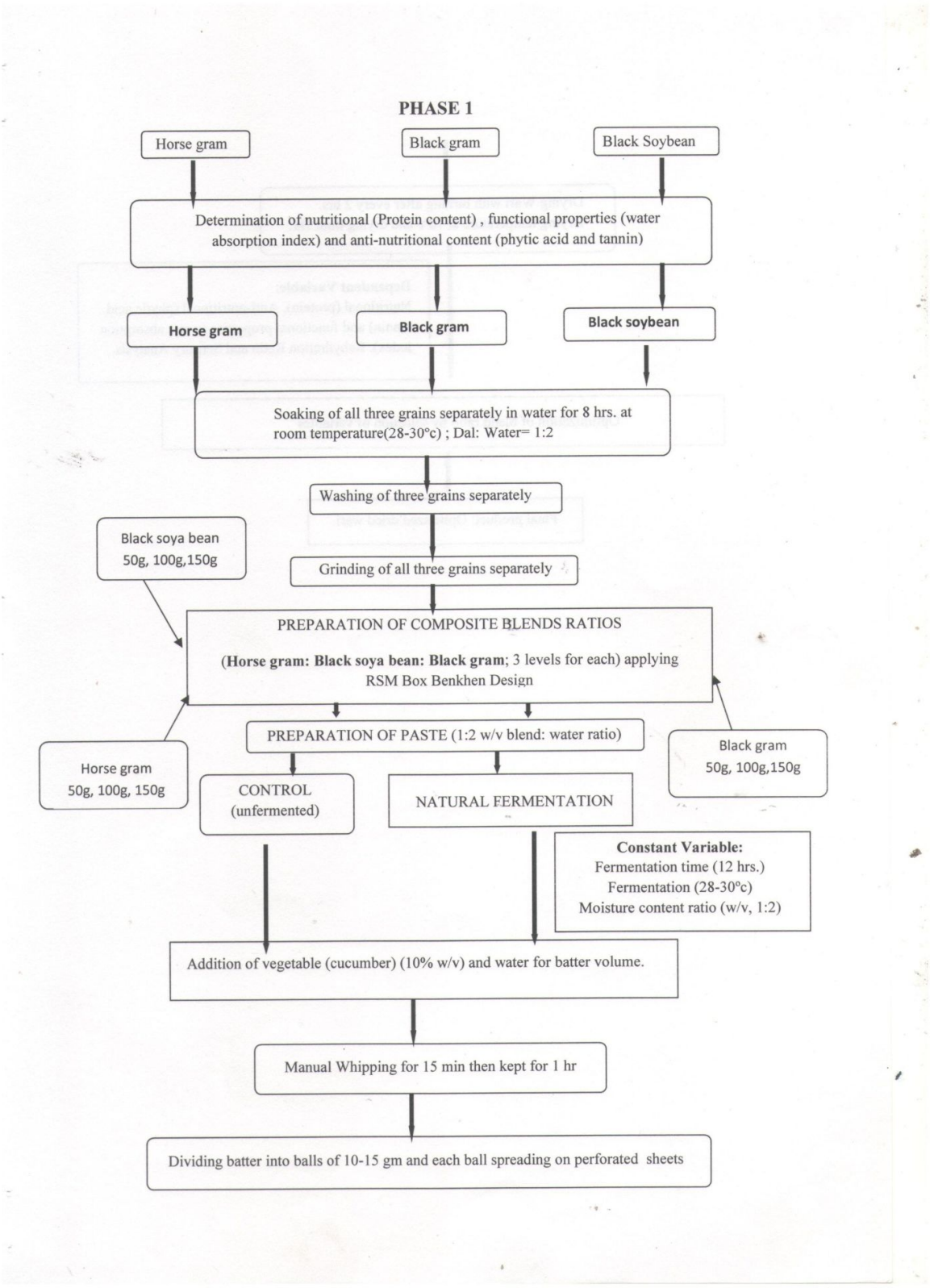
Parameter	Value/Name
Drying time	8 hrs
Drying temperature	70±1ºC
Fermentation time	12hr
Fermentation temperature	28-30 ºC (room temperature)
Moisture content (db)	8-9%
Soaking time	8 hrs.

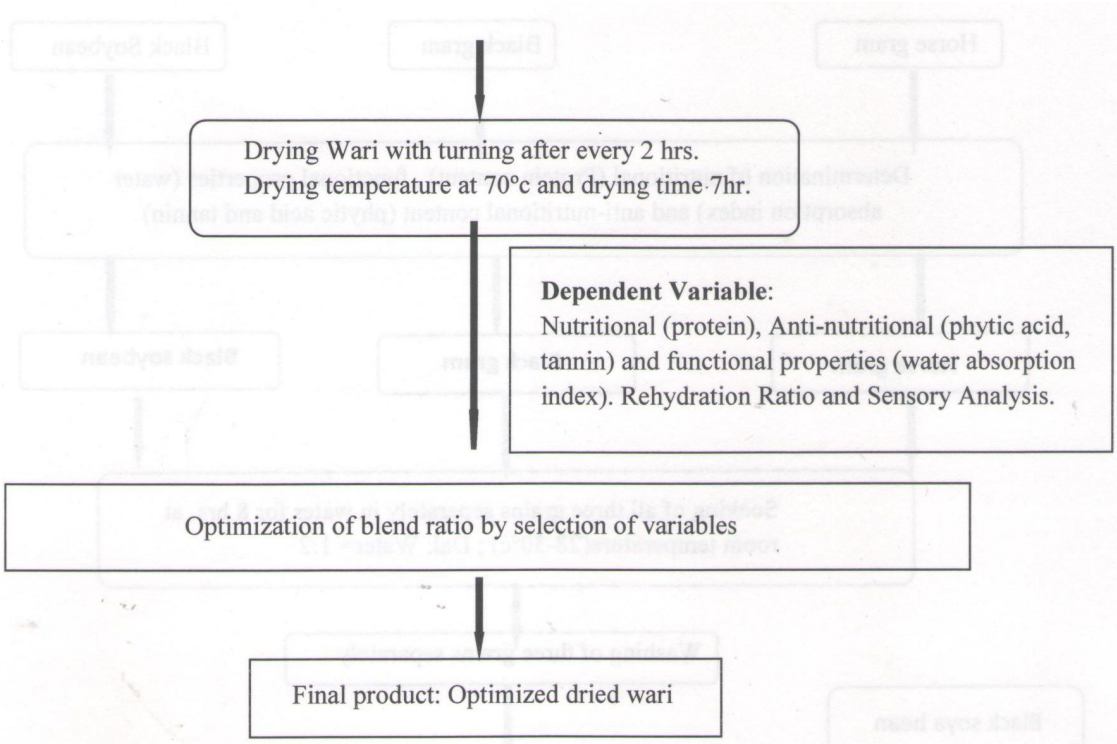
Table 3.3: Independent variables

S. No.	Parameters	Levels	Range
1.	Horse gram	3	50, 100, 150g
2.	Black soybean	3	50, 100, 150g
3.	Black gram	3	50, 100, 150g

Table 3.4: Dependent variables

S. No.	Parameter
1.	Rehydration ratio
2.	Protein
3.	Water Absorption Index (WAI)
4.	Sensory parameter
5.	Tannin
6.	Phytic acid


PHASE 1
Horse gram
Black gram
Black Soybean
Determination of nutritional (Protein content) , functional properties (water absorption index) and anti-nutritional content (phytic acid and tannin)
Horse gram
Black gram
Black soybean
Soaking of all three grains separately in water for 8 hrs. at room temperature(28-30°c) ; Dal: Water= 1:2
Washing of three grains separately
Black soya bean 50g, 100g,150g
Grinding of all three grains separately
PREPARATION OF COMPOSITE BLENDS RATIOS
(Horse gram: Black soya bean: Black gram; 3 levels for each) applying RSM Box Benkhen Design
PREPARATION OF PASTE (1:2 w/v blend: water ratio)
Horse gram 50g, 100g, 150g
Black gram 50g, 100g,150g
CONTROL (unfermented)
NATURAL FERMENTATION
Constant Variable:
Fermentation time (12 hrs.)
Fermentation (28-30°c)
Moisture content ratio (w/v, 1:2)
Addition of vegetable (cucumber) (10% w/v) and water for batter volume.
Manual Whipping for 15 min then kept for 1 hr
Dividing batter into balls of 10-15 gm and each ball spreading on perforated sheets


Drying Wari with turning after every 2 hrs.
Drying temperature at 70°c and drying time 7hr.
Dependent Variable:
Nutritional (protein), Anti-nutritional (phytic acid, tannin) and functional properties (water absorption index). Rehydration Ratio and Sensory Analysis.
Optimization of blend ratio by selection of variables
Final product: Optimized dried wari

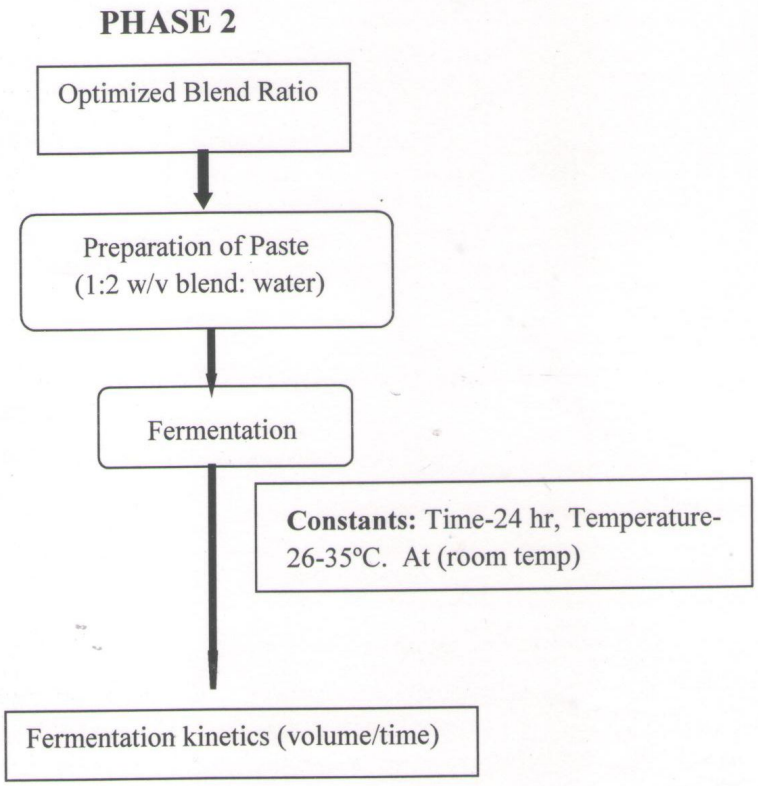


Fig. 3.1 EXPERIMENTAL PLAN

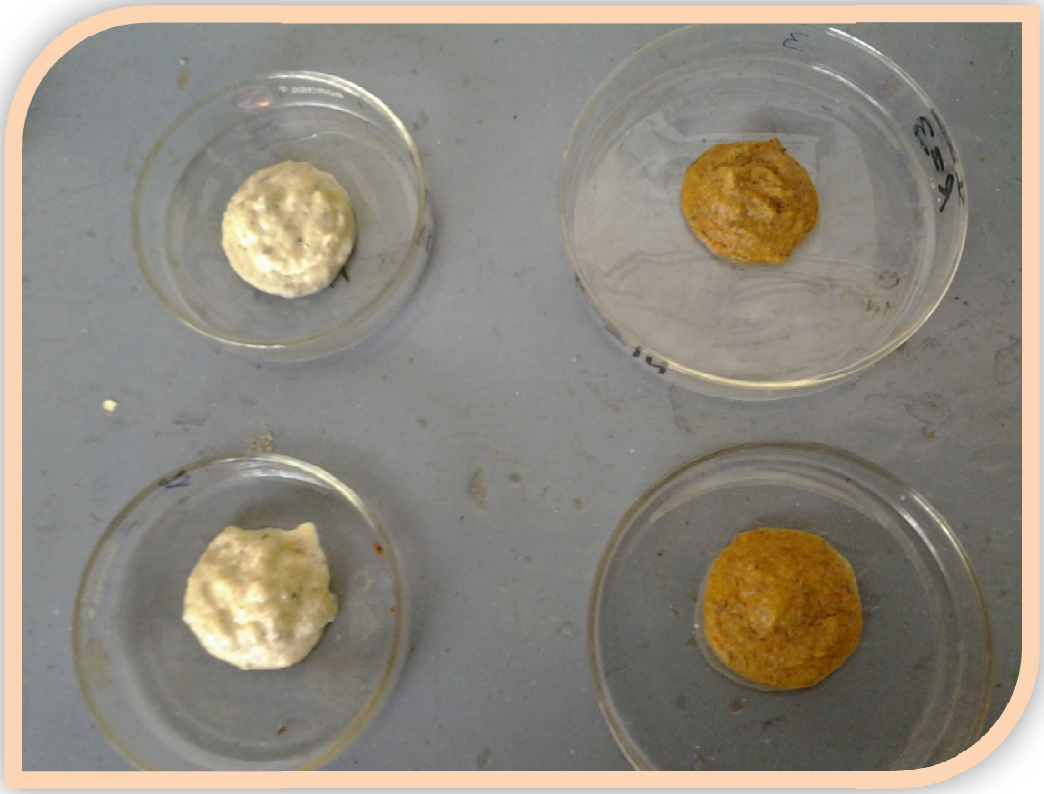


Plate 1: Fresh and dried fermented wari samples

A **B**

Plate 2: Fermented batter (showing growth kinetics)

To find out all possible combinations of parameters, experiments were designed using Response Surface Methodology using Design Expert 7.0.0 software. 17 experiments were conducted for the samples subjected to natural fermentation and 17 samples were kept as a control sample. Total numbers of experiments for the phase 1 were 34. Box benkhen design was used on three independent variables (horse gram (g), black soybean (g), black gram (g)) at three levels. The detail experimental design is given in table 3.5.

Table 3.5: Process variables & their levels

Independent variables		**Coded levels**		
Name	**Code**	**-1**	**0**	**1**
		Actual levels		
Horse gram (g)	X_1	50	100	150
Black gram (g)	X_2	50	100	150
Black soybean (g)	X_3	50	100	150

Table 3.6: Experimental design

		Coded values			**Actual values**		
Standard	**Run**	**X_1**	**X_2**	**X_3**	**Horse gram**	**Black gram**	**Black soybean**
6	1	1.00	0.00	-1.00	150	100	50
17	2	0.00	0.00	0.00	100	100	**100**
7	3	-1.00	0.00	1.00	50	100	150
5	4	-1.00	0.00	-1.00	50	100	50
1	5	-1.00	-1.00	0.00	50	50	100
4	6	1.00	1.00	0.00	150	150	100
10	7	0.00	1.00	-1.00	100	150	50
11	8	0.00	-1.00	1.00	100	50	150
12	9	0.00	1.00	1.00	100	150	150
2	10	1.00	-1.00	0.00	150	50	100
3	11	-1.00	1.00	0.00	50	150	100
16	12	0.00	0.00	0.00	100	100	100
14	13	0.00	0.00	0.00	100	100	100
15	14	0.00	0.00	0.00	100	100	100
9	15	0.00	-1.00	-1.00	100	50	50
8	16	1.00	0.00	1.00	150	100	100
13	17	0.00	0.00	0.00	100	100	100

3.4 Analytical Procedures

3.4.1 Proximate composition

Moisture content, Protein, ash, fat, fiber, carbohydrate and minerals contents were measured using standard procedures.

3.4.1.1 Moisture content

The moisture content was determined by oven dry method **(Ranganna, 1986).** 5 g finally grind sample was taken in Petri dish and the weight is recorded as W_1. The weight of empty petri dish before drying was recorded as W_0. The sample was kept in oven at 130°C for 2 h. The Petri dish was then covered with its lid and put into desiccators containing activated silica gel for cooling. When the dish cooled down to room temperature, it was weighed and its weight is recorded as W_2. The moisture content determination was carried out twice for greater accuracy. Moisture content of the sample was calculated using the following equation

$$\text{Moisture content (\% wb)} = \frac{W_1 - W_2}{W_1 - W_0}$$

Where,

W_0 = Weight of empty Petri dish, g

W_1 = Weight of sample before drying + weight of Petri dish, g

W_2 = Weight of sample after drying + weight of Petri dish, g

3.4.1.2 Ash

Total ash content was determined by **AOAC, 1984** method. 10 g of sample was taken in a dried and preweighed (W_1) silica crucible and the weight is recorded as W_2. The crucible was then ignited over heater until fumes ceased off. Ashing was done in a muffle furnace at 550 ± 5^0C until sample was become carbon free. The sample was then removed and weighed and its weight recorded as W3. Difference in weight of sample before and after ashing, expressed as % total ash as follows:

$$\% \text{ Total ash} = \frac{W_2 - W_1}{W_3 - W_1}$$

Where,

W1 = Weight of empty Petri dish, g

W2 = Weight of sample before ahing + Petri dish, g

W3 = Weight of sample after ahing + Petri dish, g

3.4.1.3 Fat

Fat content in food was determined using soxhlet extraction method **(AOAC, 1984)**. The 5 g oven dried sample was taken in thimble and plugged with fat free cotton. Then the thimble was dropped in fat extraction tube of Soxhlet apparatus. It was attached to the bottom of the extraction tube to a Soxhlet flask. Approximately 75 ml of petroleum ether was poured through the sample in the tube into flask and was attached to the top of the fat extraction tube to condenser. The sample was extracted for 16 hr on water bath. After extraction, the solvent was evaporated in oven at 100 ºC for 30 min. After cooling, the flask containing crude fat was weighted.

$$\% \text{ Crude fat} = \frac{\text{Weight of fat}}{\text{Weight of sample}}$$

3.4.1.4 Fiber

The crude fiber content in sample was estimated using **(AACC, 1996)** method. The 2 g defatted sample was digested with 200 ml of 1.25% sulphuric acid for 30 min. After filtration through a linen cloth, the residue was washed with boiled distilled water until it free from acid. The acid free residue was digested with 200 ml of 1.25% sodium hydroxide for 30 min. the content was filtrate through a linen cloth. The residue was then transfer to gooch crucible and washed with boiled distilled water until it alkali gets free. Finally the residue was washed with 15 ml of 95% ethyl alcohol. The contents of crucible were dried to a content weight at 100ºC. The dried residue was then ignited in a muffle furnace at 550ºC for 30min. The percent loss in weight was expressed as crude fiber.

3.4.1.5 Protein

Protein content in grains was determined using Lowry method **(Lowry *et al.* 1951).**

Procedure

Preparation of standard curve - Make up to 10 standard (4 or more) to concentration of 0.1- 1.0mg/ml from the standard stock solution of BSA. Dilute samples to within the range of protein concentration of the standards. A 5 ml of reagent C was added to 1 ml sample in a test tube and mixed thoroughly. It was kept at room temperature for 10 minutes then 0.5 ml of reagent D was added rapidly in each tube and mixed completely. After 30 min or longer calibrate the instrument using a blank with reagents and read at 750nm. A standard curve was developed between Bovine Serum Albumin (BSA) and optical density (fig.1 in appendix A). This curve was used to determine protein content (mg/ml).

3.4.2 Antinutritional factors

Tannin content and phytic acid was determined using standard methods as mentioned below.

3.4.2.1 Tannin estimation

Tannin content in flour was estimated by the colorimetric method as described by **(Ranganna, 2003).**

Preparation of standard curve

The varying aliquots (6-10 ml) of the standard tannic acid solution were pipetted into 100 ml volumetric flask containing 75 ml of water, and 5 ml of Folin Denis reagent. To this 10 ml of Sodium carbonate solution was added, and made up the volume to 100 ml with water. The content was mixed well and colour was measured after 30 min. at 760 nm against experimental black. The relationship of O.D. with varying amount of tannic acid in standard solution was converted into standard curve, which was used for tannin estimation.

Preparation of Sample

The 5 g of sample was boiled for 30 min. with 400 ml of water, cooled and transfer to a 500 ml of volumetric flask and diluted to mark. The content were shaken well and filtered.

Calculation

$$\% \text{ Tannic acid} = \frac{\text{mg of tannic acid} \times \text{dilution} \times 100}{\text{ml of sample taken for colour development} \times \text{Weight of sample}} \times 100$$

3.4.2.2 Phytic acid

Phytate content in the grain sample was determined by the methods of **(Wheeler and Ferrel, 1971).**

Procedure

Finely ground sample (5 g) of seed was extracted with 50 ml of 3% TCA for 45 min with occasional swirling by hand. The content was centrifuged for 15 min and supernatant collected was made to a known volume. An aliquot of 10 ml of the supernatant was transferred to a 40 ml conical centrifuge flask. Ferric chloride solution (4 ml) was added to the aliquot by blowing rapidly from the pipette. The contents were heated in a boiling water bath for 45min. After 30 min, 3-4 drops of sodium sulphate in 3 % TCA was added and heating continued. The contents were centrifuged for 15 min and the supernatant was decanted carefully. The precipitate was washed well by dispersing with 25 ml TCA, heated in boiling water bath for 10 mins, and centrifuged. Again the supernatant was decanted carefully and discarded. This process of washing the precipitate was repeated once more with TCA. The washing was again repeated with distilled water. The precipitate was dispersed with 2-3 ml of distilled water and 3 ml of NaOH was added, mixing the contents. The contents were brought to about 30 ml with distilled water heating in boiling water bath for 30 min. The contents were filtered through Whatman No.2 filter paper, quantitatively. The precipitate was washed with 100 ml of hot water. The filtrate was discarded. The precipitate on the paper was dissolved with 40 ml of hot 3.2 N nitric acid into 100ml volumetric flask. The paper

was washed with several portions of water and all the washings were collected in the same flask. The contents of the flask were cooled and volume made up with water. A known volume of aliquot of the phytate extract was pipetted into a test tube and iron content was determined using orthophenanthraline.

Phytate was calculated assuming a constant of 4Fe:6P molecular ratio in the precipitate as per the equation given below:

$$\text{phytate phosphorous } (mg.g^{-1}) = \frac{\mu g \text{ Fe x } 15}{\text{Sample weight (g)}} \times 100$$

3.4.3 Functional property

3.4.3.1 Water absorption index (WAI)

The water absorption index (WAI) of the flour was determined using procedure described by Anderson *et al* (1969). A 2.5 g sample was weighed in Nalgene centrifuge tubes, and 30 ml of distilled water was added to it. The tubes were then heated in a water bath at 95°C for 30 min and stirred with a glass rod after every 5 min. heated samples were then centrifuged at 3000 rpm for 15 min. The supernatant was decanted completely and gel weight was taken for estimation.

$$\text{WAI} = \frac{\text{Wt. of gel (g)}}{\text{Wt. of dry sample}}$$

3.4.3.2 Rehydration ratio

Rehydration of dried wari sample were carried out to test the expansion of the wari samples on reconstitution. The rehydration was done of 34 samples from each set of experiments to get the rehydration ratio of samples.

Rehydration was done using standard procedure **(Ranganna, 2003)**. Accurately weighed samples were boiled in 250 ml of water in a beaker and brought to boil. It was observed that constant weight came after 4 hr of rehydration. Sample were taken out at ½ hr, 1 hr, 2 hr and 4 hr intervals and weighed after complete removal of surface water using blotting paper.

Rehydration ratio = WR/WD

Where,

WR= weight of rehydrated wari samples, g

WD=weight of dehydrated wari samples, g

3.4.3.3 Organoleptic properties

3.4.3.3.1 Sensory analysis

Sensory quality of wari at different trials were evaluated by panelists and later the most acceptable variations were subjected to organoleptic evaluation by a panel of judges using a nine point hedonic scale. Fresh wari samples were evaluated for their crust color, crust texture, flavor (aroma), taste and overall acceptability. The evaluation sheets for the testing of rehydrated samples were used (Appendix A).

3.5 Data Analysis

Data analysis and optimization was done by using Design-Expert 7.0.0 software. Full order model was fitted in various responses and independent variables using least square regression analysis. Effect of independent variables on the responses was interpreted using the models. Contour plots were drawn with the help of SURFER 9.0 to get the range of independent variables for product development.

Results and Discussion

The present study was carried out to develop a fermented value added product (wari) from underutilized crops of Uttarakhand. For this, horse gram and black soybean were chosen and black gram was added to develop the product as black gram is mostly used for preparation of wari at house hold level.

The independent variables selected in the design of experiments were horse gram (x_1), black gram (x_2) and black soybean (x_3) having three levels each. The responses studied were protein, tannin, phytic acid, rehydration ratio, water absorption index. Sensory evaluation for the developed product was conducted based on color, taste, texture, flavor and overall acceptability.

In phase 2, fermentation was carried out for optimized wari samples (of phase 1) and fermentation kinetics was studied for the same. The effect of fermentation on the nutritional, anti nutritional characteristics of the optimized blend was also studied.

The experiments were planned using Response Surface Methodology **(Khurri and Cornell, 1987)** with Box Benkhen Design for three variables(having three levels each). A full second order model was fitted into each response.

The adequacy of the model was tested using coefficient of determination (R^2) and Fisher's F-test. The model was used to interpret the effects of variables, namely blends of horse gram, black gram and black soybean on responses viz. protein, tannin content, phytic acid, rehydration ratio,water absorption index and over all acceptability. If the model was found adequate, the best fit equations were developed in order to draw contour plots for showing the effect of independent variables on those responses and to select the range of variables for an acceptable product. Optimization of various process conditions was done using software Design-Expert 7.0.0.

The data from all the seventeen experiments were analyzed and the response functions were developed using multiple regression. ANOVA was used to analyze the models. The results obtained, their analysis and interpretaion are presented in this chapter.

4.1 Proximate composition, anti-nutritional factors and functional property of raw grains

Proximate composition (moisture, protein), anti-nutritional factors (phytic acid, tannin,) and functional property (water absorption index) were determined for raw grains.

Experimental data shows that raw black soybean grain had 8.13% moisture content on dry basis. The protein for black soybean was observed to be 41.03%. Phytic acid content and tannin content in raw black soybean was observed to be 235mg/100g , 0.240% respectively. The same results are in the range as reported by **Toledo *et al.* (2007).** Water absorption index of raw black soybean was 3.540g/g.

Raw horse gram grain had 11.25% moisture content on dry basis. Protein was 22.02% and phytic acid content was observed to be 192mg/100g where as tannin content was 0.425%. Water absorption index of raw horse gram was 6.02g/g. It is observed that the nutritional, anti- nutritional and functional properties of both the grains are in the range as reported by other researchers (**Kapoor *et al.* (1975)**, **Sunderraj and Thulasidas (1976**) and **Wolf, (2002).**

Black soybean has very high amount of protein as compare to horse gram. Anti-nutritional content in case of black soybean as compare to horse gram is very less. Horse gram has very high amount of anti- nutritional content. Similar findings has been reported by **Sunderraj and Thulasidas (1976**) **Sudha *et al.* (1995).** The data reveals that black soybean and horse gram are rich in nutrients but have anti-nutrients also. These anti- nutrient limit the use of these grains. A suitable processing technique needs to be explored which can enhance the bio availability of these nutrients.

4.2 Effect of independent variables on various responses for fermented and control wari samples

4.2.1 Protein

Effect of independent variables (horse gram, black gram and black soybean) on protein content was determined. Experiment data has been tabulated in Table 4.1 (fermented samples) and Table 4.2 (control samples).

Experimental data in Table 4.1 shows that in case of fermented wari samples, the protein content ranged from 19.35 to 25.45%. Maximum protein content (25.45%) was found in case of experiment no. 9 having horse gram 100g, black gram 150 g and black soybean 150 g. Minimum protein (19.35%) was found for the samples having horse gram 100g, black gram 150 g and black soybean 50g. Since black soybean has high amount of protein, blends showed the high values in case of samples where amount of black soybean was high.

In case of control wari sample, the protein ranged from 17.02 to 20.81%. the maximum protein (20.81%) was found in the case of experiment no.9 which had horse gram 100g, black gram 150g and black soybean 150g. Minimum protein (17.02%) was found when the sample horse gram 100g, black gram 100g and black soybean 100g.

It could easily be observed from Table 4.1 and 4.2 that fermentation process enhanced the amount of protein as for control samples it was observed to be 20.81 and for fermented sample 25.45% indicating 18.23% increase has addition of black soybean had effect on protein as maximum protein was for the samples having 150g of black soyabean.

4.2.2 Tannin

Data tabulated in Table 4.1 and 4.2 shows that tannin content ranged from 0.165 to 0.285%. Maximum tannin(0.285%) was found in case of experiment no.10 having horse gram 150g, black gram 50 g and black soybean 100g. Minimum protein (0.165%) was observed experiment no.9 and 13.

In case of control wari sample, the tannin content ranged from 0.185 to 0.315%. Maximum tannin (0.315%) was found in the case of experiment no.7 which had horse gram 100g, black gram 150g and black soybean 50g. Minimum tannin (0.185%) was found in the case of experiment no 3 having horse gram 50g, black gram 100g and black soybean 150g.

Tannin content in the samples were effected by the fermenting conditions and the quantity of grain. Tannin content in fermented sample was lower then non

fermented samples. Also varied with horse gram level. The reason for this might due to horse gram contain higher tannin content (12.12%).

4.2.3 Phytic acid

Experimental data for phytic acid are reported in Table 4.1 and Table 4.2. In case of fermented wari samples, phytic acid ranged from 104.73 to 149.62mg/100g. Maximum phytic acid (149.62mg/100g)was found in case of experiment no. 6 havihong rse gram 150g, black gram 150 g and black soybean 100 g. Minimum phytic acid (104.73 mg/100g) was observed for the samples having horse gram 50g, black gram 100g and black soybean 150g.

In case of control wari sample, the phytic acid ranged from 124.05 to 185.65 mg/100g. Maximum phytic acid (185.65 mg/100g)was found in the case of experiment no 11 which had horse gram 50g, black gram 150g and black soybean 100g. Minimum phytic acid (124.05 mg/100g) was found when the sample was had horse gram 50g, black gram 150g and black soybean 100g(for experiment no. 17).

Results revealed that fermentation process could decrease the phytic acid content as max. value of phytic acid for fermented wari samples was observed to be 149.62 and for control sample, 185.65 indicating 24.08% decrease.

4.2.4 Water absorption index

Experimental data Table 4.1 and 4.2 shows that in case of fermented wari samples, Water absorption index ranged from 3.25 to 5.15g/g. Maximum water absorption index (5.15g/g) was found in case of experiment no. 8 having horse gram 100g, black gram 50 g and black soybean 150 g. Minimum water absorption index (3.25g/g) was found for the samples having horse gram 50g, black gram 100 g and black soybean 50g.

In case of control wari sample, Water absorption index ranged from 3.25 to 4.65g/g. Maximum water absorption index (4.65g/g) was found in the case of experiment 1,5 and 11. Minimum water absorption index (3.25g/g) was found in the case of experiment no.16 having horse gram 150g, black gram 100g and black soybean 100g.

Water absorption index in the samples were effected by the fermentation conditions. Fermentation process could increase the water absoption index as maximum value of Water absorption index for the fermented wari samples was observed to be 5.15 and for control sample 4.65 indicating 9.71 % increase.

4.2.5 Rehydration ratio

Experimental data for rehydration ratio are reported in Table 4.1 and 4.2. In case of fermented wari samples, rehydration ratio ranged from 2.15 to 3.15. Maximum rehydration ratio (3.15) was found in case of experiment no. 8 having horse gram 100g, black gram 50g and black soybean 150 g. Minimum rehydration ratio (2.15) was observed for the sample(15) having horse gram 100g, black gram 50 g and black soybean 50g.

In case of control wari sample, rehydration ratio ranged from 2.05 to 2.65. Maximum rehydration ratio (2.65) was found in the case of experiment no. 11which had horse gram 50g, black gram 150g and black soybean 100g. Minimum rehydration ratio (2.05) was found when in the case of experiments no. 1, 4 and 7.

Results revealed that fermentation process could increase the rehydration ratio as maximum value of rehydration ratio for the fermented wari samples was observed to be 3.15 and for control sample, 2.65 indicating 15.9% increase.

4.2.6 Overall acceptability

Data tabulated in Table 4.1 and 4.2 shows that overall acceptibility ranged from 7.1 to 8.5. Maximum overall acceptibility (8.5) was found in case of experiment no. 8 having horse gram 100g, black gram 50 g and black soybean 150 g. Minimum overall acceptibility (7.1) was observed in case of experiment no. 1 having horse gram 150g, black gram 100 g and black soybean 50g.

In case of control wari sample, over all acceptability range from 7.2 to 8.2. Maximum overall acceptibility (8.2) was observed in the case of experiment no. 14 which had horse gram 100g, black gram 100g and black soybean 100g. Minimum overall acceptibility (7.2) was found in the case of experiment no. 2 having horse gram 100g, black gram 100g and black soybean 100g.

Result revealed that fermentation process could increase the overall acceptibility as max. value of overall acceptablity for the fermented wari samples was observed to be 8.5 and for control sample, 8.2 indicating 3.5 % increase.

4.3 Development of Second Order Model

A complete second order model (Eqn. 4.1) was fitted to the data and adequacy of the model was tested considering R^2 (the coefficient of multiple determination) and fisher's F-test. The models were then used to interpret the effect of various parameters on the response. Optimization of process parameters was carried out and contours were developed for selected parameters. Following gives the details of result and discussion.

A second order response function for three independent variables has the following general form:

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i X_i + \sum_{i=1}^{2}\sum_{j=i+1}^{3}\beta_{ij}X_i X_j + \sum_{i=1}^{3}\beta_{ii}X_i^2 \quad \ldots\ldots 4.1$$

Where,

β_0, β_{ii}, β_{ij} are constants

X_i, X_j are variables (coded)

The experimental data were then analyzed employing multiple regression techniques to develop response functions and variable parameters optimized for best outputs. The regression coefficients of complete second order model and their significance are reported in Table 4.3 and Table 4.10. The program provided the values of coefficients of model and related statistics in terms of lack of fit and p-value. The value of p represented the probability of significance. A high p-value indicated that the model had a significant lack of fit and therefore, considered to be inadequate. The lower, the values of P, better would be the model. The models having p-value lower than 0.1 (indicating the lack of fit is insignificant at 90% confidence level) were accepted.

The probability of significance of predictor's coefficient indicates the extent of effect of predictor on the response. The sign and magnitude of the coefficient explains

Table 4.1 Experimental Design for fermented samples of wari

Expt. No.	Coded Variables			Responses					
	Horse gram (g)	Black gram(g)	Black soyabean(g)	Protein%	Tannin %	Phytic Acid mg/100g	Rehydration Ratio	Over all acceptibility	WAI g/g
1	150	100	50	20.65	0.275	119.2	2.25	7.1	3.25
2	100	100	100	21.55	0.185	135.01	2.55	7.6	4.55
3	50	100	150	24.65	0.175	104.73	2.95	8.2	4.95
4	50	100	50	19.55	0.185	118.04	2.45	7.2	3.75
5	50	50	100	23.85	0.205	113.19	2.65	7.8	4.65
6	150	150	100	23.65	0.215	149.62	2.85	7.8	4.2
7	100	150	50	19.35	0.195	123.59	2.35	7.7	3.65
8	100	50	150	25.25	0.175	106.2	3.15	8.5	5.15
9	100	150	150	25.45	0.165	142.31	3.05	8.2	5.05
10	150	50	100	23.65	0.285	120.37	2.65	7.3	3.35
11	50	150	100	23.65	0.215	127.91	2.65	7.5	4.05
12	100	100	100	20.75	0.195	123.5	2.35	7.5	4.35
13	100	100	100	22.15	0.165	125.52	2.25	7.6	4.45
14	100	100	100	21.35	0.185	124.22	2.35	7.5	4.15
15	100	50	50	20.15	0.245	131.52	2.15	7.3	3.55
16	150	100	100	25.15	0.255	128.18	2.95	7.7	3.85
17	100	100	100	22.25	0.205	124.04	2.45	7.6	4.35

Table 4.2: Experimental Design for control samples of wari

Expt. No.	Coded Variables			Responses					
	Horse gram (g)	Black gram(g)	Black soyabean(g)	Protein %	Tannin %	Phytic Acid mg/100g	Rehydration Ratio	Over all acceptibility	WAI g/g
1	150	100	50	18.07	0.235	149.05	2.05	7.5	4.65
2	100	100	100	17.02	0.285	135.05	2.15	7.2	4.55
3	50	100	150	20.03	0.185	124.65	2.55	7.9	4.05
4	50	100	50	18.15	0.235	128.05	2.05	7.7	3.75
5	50	50	100	19.43	0.225	145.35	2.25	7.8	4.65
6	150	150	100	19.63	0.255	144.65	2.35	7.4	4.2
7	100	150	50	18.25	0.315	133.55	2.05	7.5	3.65
8	100	50	150	20.04	0.235	126.25	2.45	7.8	3.85
9	100	150	150	20.81	0.215	142.35	2.55	8.1	4.05
10	150	50	100	19.42	0.265	160.75	2.45	7.3	3.35
11	50	150	100	19.01	0.215	185.65	2.65	7.5	4.65
12	100	100	100	18.94	0.235	133.25	2.35	7.5	4.35
13	100	100	100	19.07	0.195	145.45	2.25	7.2	4.05
14	100	100	100	18.92	0.195	144.25	2.35	8.2	4.15
15	100	50	50	17.73	0.245	131.54	2.15	7.3	3.55
16	150	100	100	20.35	0.235	158.25	2.45	7.5	3.25
17	100	100	100	19.06	0.225	124.05	2.15	7.6	4.35

the nature of the effect. Negative sign at linear level means decrease in response when the level of the predictor is increased while positive sign indicates increase in the response. Significant negative interaction suggests that the level of one of the predictors can be increased while that of other decreases for constant value of the response.

Positive interaction means that the response is minimum at centre point and it increases with increase or decrease of both the variables from centre point. Positive coefficient of a quadratic term indicates the minimum response at centre value of the parameter and it increases with increase or decrease in parameter level. Negative coefficient of the quadratic term shows the maximum response at the centre value and it decreases with increase/decrease in parameter level.

4.4 Effect of independent variables on different responses

During the fermentation process, each response got affected by independent variables. It was determined by analysis using Design expert 7.0.0. ANOVA for each responses (WAI, rehydration ratio, over all acceptability, protein, tannin content and phytic acid) was done and discussed in the following sub heading. The result of regression analysis for dependent parameters are reported in Table 4.3.

4.4.1 Effect of blend ratios on protein content

The protein content was determined for each experiment (as per experimental runs given in Table 4.1 for fermented samples) with respect to independent variables. Experiment data for the same has been reported in Table 4.2 (control sample). Full second order model, Eqn. 4.2 was fitted into the protein and various experimental conditions using multiple regression analysis. Effect of independent variables on protein at linear, quadratic and interactive level is reported in Table 4.4 Total effect of individual parameter on protein was calculated using the sequential sum of squares, and it is given in the Table 4.5. The protein during fermentation was found to be in the range of 19.35 to 25.45%.

The maximum protein (25.45%) indicates the experimental conditions where sapmle (9) was having horse gram (X_1= 100g), black gram (X_2 = 150g) and black

soybean (X_3 = 150g) while the minimum protein was for the sample (7) having horse gram (X_1 = 100g), black gram (X_2 = 150g) and black soybean (X_3 = 50g).

The coefficient of determination (R^2) for the regression model for protein was 96.62%, which implies that the model could account for 96.62% data. Model was highly significant ($p < 0.01$) with F value of 22.22. Therefore, second order model was found to be adequate in describing change in protein content.

$$Y = 21.61 + 0.18X_1 - 0.10X_2 + 2.60X_3 + 0.050X_1X_2 - 0.15X_1X_3 + 0.25X_2X_3 + 1.02X_1^2 + 1.07X_2^2 - 0.13X_3^2 \quad \ldots 4.2$$

where,

Y = protein content of fermented sample

X_1, X_2 and X_3 are coded variables of horse gram, black gram and black soyabean.

Effect of independent variables on protein at linear, quadratic and interactive level is reported in Table 4.4.

Table 4.3 shows that the effect was highly significant ($p < 0.01$) at linear level and quadratic level. The model F- value was also found to be highly significant at 1% level of significance. Lack of fit was insignificant; therefore second order model was adequate in describing protein content.

Total individual parameter on protein was calculated using the sequential sum of squares, and it is given in the Table 4.5. It was observed that only black soybean affected the protein at 1% level of significance and black gram and horse gram at 10% level of significance.

4.4.2 Effect of blend ratios on tannin content

Tannin content was determined for various experimental conditions with respect to horse gram, black gram and black soybean. Experimental data of tannin content is reported in Table 4.1. shows that tannin content during fermentation ranged from 0.165 to 0.285% of sample. The maximum tannin content i.e., 0.285% was found for the experiment No 10 having horse gram (X_1= 150), black gram (X_2 = 50) and black soybean (X_3 = 100). The minimum tannin content 0.165% was found in the experiment No 9 having horse gram (X_1 = 100), black gram (X_2 = 150) and black soybean (X_3 = 150).

Table 4.3: Result of Regression analysis for protein, tannin and phytic acid for fermented samples

Source	Protein		Tannin		Phytic acid	
	Coeff.	P Value	Coeff.	P Value	Coeff.	P Value
Model	21.61	0.0002	0.19	0.0136	126.46	0.0018
X_1	0.18	0.4123	0.032	0.0023	6.68	0.0031
X_2	-0.10	0.6337	-0.015	0.0598	9.02	0.0006
X_3	2.6	< 0.0001	-0.016	0.0454	-1.36	0.3992
$X_1 X_2$	0.050	0.8652	-0.020	0.0723	3.63	0.1329
$X_1 X_3$	-0.15	0.6136	-0.0025	0.7992	5.59	0.0346
$X_2 X_3$	0.25	0.4078	0.010	0.3255	11.01	0.0013
X_1^2	1.02	0.0078	0.035	0.0065	-3.54	0.1333
X_2^2	1.07	0.0062	0.0077	0.4283	4.85	0.0527
X_3^2	-0.13	0.6528	.0002	0.9791	-5.40	0.0357
R^2	96.62%		88.56%		93.85%	
F value	22.22		6.02		11.86	
LOF	NS		NS		NS	

Table 4.4: ANOVA for protein content for fermented samples

SOURCE	DF	SS	MS	F-Value
Model	9	64.47	7.16	22.22***
Linear	3	54.41	18.13	56.65***
Interactive	3	0.35	0.11	0.34
Quadratic	3	9.91	3.39	10.59***
Error	7	2.26	0.32	
Total	16	66.73		

***, **,* Significant at 1, 5 and 10 % level of significance respectively

$F_{tab}(9,7)=2.72$; $F_{tab}(3,7)=3.07$ (10%)

$F_{tab}(9,7)=3.68$; $F_{tab}(3,7)=4.35$ (5%)

$F_{tab}(9,7,)=6.72$; $F_{tab}(3,7,)=8.45$ (1%)

Table 4.5: Total effect of individual parameter on protein content for fermented samples

SOURCE	DF	SS	MS	F-Value
Model	9	64.47	7.16	22.22***
Horse gram (X_1)	4	4.73	1.182	3.69*
Black gram (X_2)	4	5.16	1.29	4.03*
Black soybean (X_3)	4	54.49	13.62	42.56***
Error	7	2.26	0.32	
Total	16	66.73		

***, **,* Significant at 1, 5 and 10 % level of significance respectively

$F_{tab}(9,7)= 2.72$; $F_{tab}(4,7)=2.96$ (10%)

$F_{tab}(9,7)=3.68$; $F_{tab}(4,7)=4.12$ (5%)

$F_{tab}(9,7)=6.72$; $F_{tab}(4,7)=7.85$(1%)

Full second order model, Eqn. 4.3 was fitted into the tannins and various experimental conditions using multiple regression analysis. Results obtained are given in Table 4.3. The coefficient of determination (R^2) for the regression model for this parameter was 88.56%, which implies that the model could account for 88.56% data. Model was highly significant ($p < 0.05$) with F value of 6.02g. Therefore, second order model was found to be adequate in describing change in tannin content.

$$Y = 0.19 + .031X_1 - 0.015X_2 - 0.016X_3 - 0.020X_1X_2 - 0.0025X_1X_3 + 0.010X_2X_3 + 0.035X_1^2 + 0.0077X_2^2 + 0.0002X_3^2 \quad \ldots 4.3$$

where,

Y = tannins content of fermented samples

X_1, X_2 and X_3 are coded variables of horse gram, black gram and black soybean.

Effect of independent variables on tannin contents at linear, quadratic and interactive level is reported in Table 4.6.

Table 4.3 shows that the effect was highly significant ($p < 0.01$) at linear level. The effect was also found to be significant ($p < 0.05$) at Quadratic level. The model

F- value was also found to be highly significant at 1% level of significance. Lack of fit was insignificant; therefore second order model was adequate in describing tannin.

Total effect of individual parameter on tannin contents was calculated using the sequential sum of squares, and it is given in the Table 4.7. It was observed that black gram affected the responses (for tannin) at 5% level of significance and effect of horse gram and black soybean was observed to be non- significant.

Table 4.6: ANOVA for tannin content for fermented samples

SOURCE	DF	SS	MS	F-Value
Model	9	0.019	0.002	6.02**
Linear	3	0.0117	0.003	13***
Interactive	3	0.002	0.0006	2
Quadratic	3	0.0054	0.0018	6**
Error	7	0.002	0.0003	
Total	16	0.021		

***, **,* Significant at 1, 5 and 10 % level of significance respectively
$F_{tab}(9,7)=2.72$; $F_{tab}(3,7)=3.07$ (10%)
$F_{tab}(9,7)=3.68$; $F_{tab}(3,7)=4.35$ (5%)
$F_{tab}(9,7)=6.72$; $F_{tab}(3,7)=8.45$ (1%)

Table 4.7 Total effect of individual parameter on tannin for fermented samples

SOURCE	DF	SS	MS	F-Value
Model	9	0.019	0.002	6.02**
Horse gram (X_1)	4	0.0146	0.0036	1.2
Black gram (X_2)	4	0.0040	0.001	3.33*
Black soybean (X_3)	4	0.0023	0.0006	2
Error	7	0.0025	0.0003	
Total	16	0.021		

***, **,* Significant at 1, 5 and 10 % level of significance respectively
$F_{tab}(9,7)= 2.72$; $F_{tab}(4,7)=2.96$ (10%)
$F_{tab}(9,7)=3.68$; $F_{tab}(4,7)=4.12$ (5%)
$F_{tab}(9,7)=6.72$; $F_{tab}(4,7)=7.85$(1%)

4.4.3 Effect of blend ratios on phytic acid

The phytic acid ranged from 106.2 to 149.62 mg/100g as tabulated in Table 4.1. Maximum phytic acid (149.62 mg/100g) content was observed for experiment no. 6 having horse gram (X_1= 100), black gram(X_2 = 50) and black soybean (X_3 = 150). Minimum phytic acid was (for expt. 8) at horse gram(X_1 = 150), black gram(X_2 = 150) and black soybean (X_3 = 100).

Full second order model, Eqn. 4.4 was fitted to the total sugar values and various experimental conditions using multiple regression analysis. Results obtained are given in Table 4.3. The coefficient of determination (R^2) for the regression model for this parameter was 93.85%, which implies that the model could account for 93.85% data. Model was highly significant ($p < 0.01$) with F value of 11.86. Therefore, second order model was found to be adequate in describing change in total sugar.

$$Y = 126.46 + 6.68X_1 + 9.02X_2 - 1.36X_3 + 3.63X_1X_2 + 5.59X_1X_3 + 11.01X_2X_3 - 3.54X_1^2 + 4.85X_2^2 - 5.4X_3^2 \quad \ldots. 4.4$$

where,

Y = phytic acid of fermented samples

X_1, X_2 and X_3 are coded variables of horse gram, black soybean and black gram.

Effect of independent variables on phytic acid at linear, quadratic and interactive level is reported in Table 4.8.

Table 4.3 shows that the effect was highly significant ($p < 0.01$) at linear , quadratic and interactive level. The model F- value was also found to be highly significant at 1% level of significance. Lack of fit was insignificant; therefore second order model was adequate in describing total sugar.

Total effect of individual parameter on phytic acid was calculated using the sequential sum of squares, and it is given in the Table 4.9. It was observed that horse gram, black gran and black soybean affected the phytic acid at 1% level of significance.

Table 4.8: ANOVA for phytic acid for fermented samples

SOURCE	DF	SS	MS	F-Value
Model	9	1949.80	216.64	11.86***
Linear	3	1022.13	340.71	18.65***
Interactive	3	662.76	220.92	12.09***
Quadratic	3	274.7	91.56	5.01**
Error	7	127.085	18.26	
Total	16	2076.89		

***, **,* Significant at 1, 5 and 10 % level of significance respectively

$F_{tab}(9,7)=2.72$; $F_{tab}(3,7)=3.07$ (10%)

$F_{tab}(9,7)=3.68$; $F_{tab}(3,7)=4.35$ (5%)

$F_{tab}(9,7)=6.72$; $F_{tab}(3,7)=8.45$ (1%)

Table 4.9: Total effect of individual parameter on phytic acid for fermented samples

SOURCE	DF	SS	MS	F-Value
Model	9	1949.80	216.64	11.86***
Horse gram (X_1)	4	587.25	146.81	8.04***
Black gram (X_2)	4	1287.44	321.86	17.62***
Black soybean (X_3)	4	747.6	186.9	10.23***
Error	7	127.85	18.26	
Total	16	2076.89		

***, **,* Significant at 1, 5 and 10 % level of significance respectively

$F_{tab}(9,7)= 2.72$; $F_{tab}(4,7)=2.96$ (10%)

$F_{tab}(9,7)=3.68$; $F_{tab}(4,7)=4.12$ (5%)

$F_{tab}(9,7)=6.72$; $F_{tab}(4,7)=7.85$(1%)

4.4.4 Effect of blend ratios on rehydration ratio

Rehydration ratio during fermentation ranged from 2.15 to 3.15 (Table 4.1) of sample over entire experimental conditions. Maximum rehydration ratio was observed at Experiment No. 8 having blend ratio of horse gram(X_1= 100g), black gram (X_2 = 50g) and black soybean (X_3 = 150g). Minimum protein content was observed for the samples of experiment no. 15 having blend ratio of horse gram (X_1 = 100g), black gram (X_2 = 50g) and black soybean (X_3 = 50g). The significance of independent variables viz., horse gram, black gram and black soybean on rehydration ratio was tested using ANOVA and total effect of individual parameters on protein was observed using ANOVA. The details are reported in Table 4.10.

Full second order model, Eqn. 4.5 was fitted into the protein and various experimental conditions using multiple regression analysis. Results obtained are given in Table 4.12. The coefficient of determination (R^2) for the regression model for this parameter

was 93.04%, which implies that the model could account for 93.04% data. Model was highly significant ($p < 0.01$) with F value of 10.40. Therefore, second order model was found to be adequate in describing change in reducing sugar.

$$Y = 2.39 + 0.00X_1 + 0.038X_2 + 0.36X_3 + 0.050X_1X_2 + 0.05X_1X_3 - 0.075X_2X_3 + 0.14X_1^2 + 0.17X_2^2 + 0.12X_3^2 \quad \ldots 4.5$$

Table 4.10 ANOVA for rehydration ratio for fermentation samples

SOURCE	DF	SS	MS	F-Value
Model	9	1.40	0.16	10.40***
Linear	3	1.061	0.35	23.33***
Interactive	3	0.043	0.014	0.95
Quadratic	3	0.264	0.088	5.86**
Error	7	0.10	0.015	
Total	16	1.5		

***, **,* Significant at 1, 5 and 10 % level of significance respectively
$F_{tab}(9,7)=2.72$; $F_{tab}(3,7)=3.07$ (10%)
$F_{tab}(9,7)=3.68$; $F_{tab}(3,7)=4.35$ (5%)
$F_{tab}(9,7)=6.72$; $F_{tab}(3,7)=8.45$ (1%)

Table 4.11: Total effect of individual parameter on rehydration ratio during fermentation

SOURCE	DF	SS	MS	F-Value
Model	9	1.40	0.16	10.40***
Horse gram (X_1)	4	0.106	0.026	1.76
Black gram (X_2)	4	0.141	0.035	2.35
Black soybean (X_3)	4	0.141	0.285	19.01***
Error	7	0.30	0.015	
Total	16	1.5		

***, **,* Significant at 1, 5 and 10 % level of significance respectively

$F_{tab}(9,7)= 2.72$; $F_{tab}(4,7)=2.96$ (10%)

$F_{tab}(9,7)=3.68$; $F_{tab}(4,7)=4.12$ (5%)

$F_{tab}(9,7)=6.72$; $F_{tab}(4,7)=7.85$(1%)

Table 4.12: Result of Regression analysis for rehydration ratio, overall acceptability and water absorption index for fermented samples

Source	Rehydration ratio		Over all acceptance		Water absoption index	
	Coeff.	P Value	Coeff.	P Value	Coeff.	P Value
Model						
X_1	0.00	1.0000	-.100	0.0050	-0.34	0.0022
X_2	0.038	0.4141	.037	0.1746	0.031	0.6828
X_3	0.36	< 0.0001	.41	< 0.0001	0.60	< 0.0001
$X_1 X_2$	0.050	0.4401	.20	0.007	0.36	0.0101
$X_1 X_3$	0.050	0.4401	-.100	0.0247	-.0.15	0.1913
$X_2 X_3$	-.075	0.2592	-.18	0.0016	-0.050	0.6444
X_1^2	0.14	0.0479	-.17	0.0018	-0.35	0.0100
X_2^2	0.17	0.0260	.21	0.0005	0.046	0.6611
X_3^2	0.12	0.0891	.16	0.0025	-0.066	0.5331
R^2	93.04%		98.43%		94.34%	
F value	10.40		48.87		12.96	
LOF	NS		NS		NS	

where,

Y = rehydration ratio of fermented samples

X_1, X_2 and X_3 are coded variables of horse gram, black gram and black soybean.

Effect of independent variables on protein at linear, quadratic and interactive level is reported in Table 4.10.

Table 4.12 shows that the effect was highly significant ($p < 0.01$) at linear level and ($p < 0.05$) at quadratic levels. The model F- value was also found to be highly significant at 1% level of significance. Lack of fit was insignificant; therefore second order model was adequate in describing reducing sugar.

Total effect of individual parameter on rehydration ratio was calculated using the sequential sum of squares, and it is given in the Table 4.11. It was observed that only black soybean affected the rehydration ratio at 1% level of significance while there was no effect of black gram and horse gram.

4.4.5 Effect of blend ratios on Overall acceptability fermented samples

Over all acceptability of samples during fermentation ranged from 7.1 to 8.5 over entire experimental conditions. Maximum O.A.A. was observed for Experiment No. 8 having blend ratios of horse gram (X_1= 100g), black gram (X_2 = 50g) and black soybean (X_3 = 150g). Minimum O.A.A. was (for expt. 1) for blend ratio having horse gram (X_1 = 150g), black gram (X_2 = 100g) and black soybean (X_3 = 50g).

The significance of independent variables viz., horse gram, black gram and black soybean on over all acceptability was tested using ANOVA and total effect of individual parameters on over all acceptability was also assessed using ANOVA. The details are given in Table 4.13.

Full second order model, Eqn. 4.6 was fitted to the overall acceptability and various experimental conditions using multiple regression analysis. Results obtained are given in Table 4.12. The coefficient of determination (R^2) for the regression model for this parameter was 98.43%, which implies that the model could account for 98.43% data. Model was highly significant at 1% level of significance with F value of 48.87.

Therefore, second order model was found to be adequate in describing change in overall acceptability.

$$Y = 7.56 - 0.1X_1 + 0.037X_2 + 0.41X_3 + 0.20X_1X_2 - 0.10X_1X_3 - 0.18X_2X_3 - 0.17X_1^2 + 0.21X_2^2 + 0.16X_3^2 \quad \ldots 4.6$$

where,

Y = over all acceptability of fermented samples

X_1, X_2 and X_3 are coded variables of horse gram, black gram and black soybean.

Effect of independent variables on over all acceptability at linear, quadratic and interactive level is reported in Table 4.13.

Table 4.12 shows that the effect was highly significant ($p < 0.01$) at linear , quadratic and interactive level. The model F- value was also found to be highly significant at 1% level of significance. Lack of fit was insignificant; therefore second order model was adequate in describing pH.

Total effect of individual parameter on overall acceptability was calculated using the sequential sum of squares, and it is given in the Table 4.14. It was observed that horse gram, black gram and black soybean affected the overall acceptability at 1% level of significance .

Table 4.13 ANOVA for Overall acceptability for fermented samples

SOURCE	DF	SS	MS	F-Value
Model	9	2.17	0.24	48.87***
Linear	3	1.45	0.48	98.70***
Interactive	3	0.32	0.106	21.76***
Quadratic	3	0.40	0.13	27.21***
Error	7	0.034	-0.0049	
Total	16	2.2		

***, **,* Significant at 1, 5 and 10 % level of significance respectively

$F_{tab}(9,7)=2.72$; $F_{tab}(3,7)=3.07$ (10%)

$F_{tab}(9,7)=3.68$; $F_{tab}(3,7)=4.35$ (5%)

$F_{tab}(9,7)=6.72$; $F_{tab}(3,7)=8.45$ (1%)

Table 4.14: Total effect of individual parameter on Overall acceptability for fermented samples

SOURCE	DF	SS	MS	F-Value
Model	9	2.17	0.24	48.87***
Horse gram (X_1)	4	0.40	0.10	20.40***
Black gram (X_2)	4	0.471	0.110	24.03***
Black soybean (X_3)	4	1.62	0.405	82.65***
Error	7	0.034	0.0049	
Total	16	2.2		

***, **,* Significant at 1, 5 and 10 % level of significance respectively

$F_{tab}(9,7)= 2.72$; $F_{tab}(4,7)=2.96$ (10%)

$F_{tab}(9,7)=3.68$; $F_{tab}(4,7)=4.12$ (5%)

$F_{tab}(9,7)=6.72$; $F_{tab}(4,7)=7.85$(1%)

4.4.6 Effect of blend ratios on Water absorption index

WAI is one of the important parameter for determining the functional parameters of grains. WAI was determined for treated and untreated grains and effect of process variables was determined by finding out the nutritional, anti nutritional and functional property of grains. The values for same have been reported in Table 4.1. Values for WAI varied from 3.25 to 5.15 g/g. The maximum value for WAI (5.15 g/g) was observed for Experiment No. 8 having horse gram (X_{1g}= 100), black gram (X_2 = 50g) and black soybean (X_3 = 150g). Minimum wai was observed for Experiment No.1 having horsegram (X_1 = 150g), black gram (X_2 = 100g) and black soybean (X_3 = 50g).

Full second order model, Eqn. 4.7 was fitted to check the validity of model using multiple regression analysis. Results obtained are tabulated in Table 4.12. The coefficient of determination (R^2) for the regression model for this parameter was 94.34%, which implies that the model could account for 94.34% data. Model was highly significant ($p < 0.01$) with F value of 12.96. Therefore, second order model was found to be adequate in describing the pattern of non-reducing sugar content.

$$Y = 4.37 - 0.34X_1 + 0.031X_2 + 0.60X_3 + 0.36X_1X_2 - 0.15X_1X_3 - 0.05X_2X_3 - 0.35X_1^2 + 0.046X_2^2 - 0.066X_3^2 \quad \text{....4.7}$$

where,

Y = water absorption index of fermented samples

X_1, X_2 and X_3 are coded variables of horse gram, black gram and black soybean. Effect of independent variables on phytic acid at linear, quadratic and interactive level is reported in Table 4.15.

Table 4.12 shows that the effect was highly significant ($p < 0.01$) at linear level only. The model F- value was also found to be highly significant at 1% level of significance. Lack of fit was insignificant; therefore second order model was adequate in describing non reducing sugar.

Total effect of individual parameter on water absorption index was calculated using the sequential sum of squares, and it is reported in the Table 4.16. It was observed that horse gram and black soybean affected the water absorption index at 1% level of significance. There was 10% effect of black gram on change in water absorption index.

4.5 Optimization of blend ratios

The objective of the study was to get the optimized blend ratio for development of wari using horse gram, black soybean and black gram. The optimized condition could be a single point or a range of points in which all the possible combinations would yield good results. While using any optimization technique some constraints have to be decided, keeping in view the optimized conditions are obtained. These constraints set the guidelines to get the desired results. The response values and the analysis of the models give the valuable information in deciding the constraints for independent variables and responses. The optimization for each dependent parameter was done by visualizing the response surface.

Table 4.15: ANOVA for water absorption index for fermented samples

SOURCE	DF	SS	MS	F-Value
Model	9	5.02	0.56	12.96***
Linear	3	3.8378	1.27	29.75***
Interactive	3	0.6300	0.21	4.88**
Quadratic	3	0.5570	0.185	4.32*
Error	7	0.30	0.043	
Total	16	5.32		

***, **,* Significant at 1, 5 and 10 % level of significance respectively

$F_{tab}(9,7)=2.72$; $F_{tab}(3,7)=3.07$ (10%)

$F_{tab}(9,7)=3.68$; $F_{tab}(3,7)=4.35$ (5%)

$F_{tab}(9,7)=6.72$; $F_{tab}(3,7)=8.45$ (1%)

Table 4.16: Total effect of individual parameter on Water absorption index on fermented samples

SOURCE	DF	SS	MS	F-Value
Model	9	5.02	0.56	12.96***
Horse gram (X_1)	4	2.10	0.525	12.209***
Black gram (X_2)	4	0.5568	0.1392	3.23*
Black soybean (X_3)	4	2.998	0.749	17.43***
Error	7	0.30	0.043	
Total	16	5.32		

***, **,* Significant at 1, 5 and 10 % level of significance respectively

$F_{tab}(9,7)= 2.72$; $F_{tab}(4,7)=2.96$ (10%)

$F_{tab}(9,7)=3.68$; $F_{tab}(4,7)=4.12$ (5%)

$F_{tab}(9,7)=6.72$; $F_{tab}(4,7)=7.85$(1%)

One of the techniques used to visualize the response surface graphically is to plot the contours of the response surface equation (Eqn. 4.1) in a contour plot , lines or curves of the constants response values are drawn on a plane or graph whose coordinate axes represent the levels of independent variables and the response is visualized perpendicular to the plane of paper. Series of contour line of equal response value were generated which provided useful information for understanding the effect of two independent parameters on the dependent variable.

4.5.1 Numerical optimization of blend ratios

Optimizatiom is a process of making compromises between responses, to achieve a common target. Numerical optimization was carried out using Design-Expert 7.0.0 statistical software. The goal was fixed to maximize the protein content and to minimize the anti-nutritional factors. The responses namely protein, tannin, phytic acid rehydration ratio, water absorption index and overall acceptability activity were taken in to consideration for optimization. The goal seeking begins at a random starting point and proceeds up and down the steepest slope on the response surface for a maximum or minimum value of the response respectively. Importance to the responses ad independent variables were given on the basis of objectives of the study. Maximum importance (++++) was given to protein, tannin, phytic acid and trypsin inhibitor activity and for the remaining responses, the goal was kept at in range. The goal setup for optimization of variables and responses is reported in Table 4.17.

Based upon mentioned criteria, the optimization was carried out. During optimization, 22 solutions were obtained in case of fermentation and 18 solution were obtained in case of control fermentation of wari paste out of which the one that suited the criteria most was selected. The most suitable optimum point is given in the Table 4.18.

The optimum level of variables in coded and actual form for fermented wari and control wari were reported in Table 4.17 and 4.18, respectively.

Table 4.19 and 4.20 reveals that optimum blend ratios for fermented and control wari are 63.5:50:150, 50:79.5:150.

Table 4.17: Constraints for optimization for fermented wari

Name	Goal	Lower limit	Upper limit
Black gram	Is in range	-1	+1
Black soybean	Is in range	-1	+1
Horse gram	Is in range	-1	+1
Rehydration ratio	Maximum	2.15	3.15
Water absorption index	Maximum	3.25	5.15
Protein	Maximum	19.35	25.45
Tannins	Minimum	0.165	0.285
Phytic acid	Minimum	149.62	104.73
Overall acceptability	Maximum	7.1	8.5

Table 4.18: Constraints for optimization for control wari

Name	Goal	Lower limit	Upper limit
Black gram	Is in range	-1	+1
Black soyabean	Is in range	-1	+1
Horse gram	Is in range	-1	+1
Rehydration ratio	Maximum	2.15	2.65
Water absorbtion index	Maximum	3.25	4.65
Protein	Maximum	17.02	20.65
Tannins	Minimum	0.185	0.315
Phytic acid	Minimum	124.05	185.65
Overall acceptability	Maximum	7.2	8.1

Table 4.19: Optimum values of blend ratios for fermented wari

Value	Horse gram(g) (X_1)	Black gram(g) (X_2)	Black soybean (g) (X_3)
Coded	-0.73	-1	+1
Actual	63.5	50	150

Table 4.20: Optimum values of blend ratios for control wari

Value	Horse gram(g) (X_1)	Black gram(g) (X_2)	Black soybean (g) (X_3)
Coded	-1	-0.41	+1
Actual	50	79.5	150

4.5.1.1 Quality of prepared wari sample

The wari was prepared using fermented blends and control blends. The results obtained from the chemical, functional analysis and the physical properties of wari are shown in Table 4.21 and 4.22 respectively. Fermentation greatly affected the physicochemical quality of wari.

4.5.1.2 Proximate composition

The proximate composition of wari prepared from fermented blend, is presented in Table 4.21. Variation in proximate compositions of fermented blend and control blend can be seen from Table 4.21. The proximate values for protein and rehydration ratio, water absorption index were lowest in control blend samples and highest in fermented wari samples. Whereas tannin content, phytic acid were observed to be high in case of control samples. After fermentation, anti-nutrition content were observed to be decreased, showing significant effect of fermentation.

Table 4.21: Proximate composition of optimized control and fermented blend samples

Optimized wari samples	% Protein	% tannin	Phytic acid mg/100gm	WAI	Rehydration Ratio g/g	O.O.A.
Fermented wari	25.5	0.166	96.03	5.1	3.1	8.6
Control wari	20.1	0.185	126.3	4.5	2.45	8.01

The effect of fermentation was found very effective as protein, WAI, overall acceptability and rehydration ratio increased by 21.18, 16.67, 6.86 and 20.97% , anti-nutritional factors, viz., tannin and Phytic acid were found to be reduced by 11.45 and 31.5%.

4.5.1.3 Sensory evaluation for optimized blend ratios (control and fermented)

Results of sensory evaluation (on 9 point hedonic scale) of wari sample containing different level of substitution as compared to the control are shown in Table 4.24. The significant difference in the fermented wari sample and the control sample was found out.

Table 4.22: Sensory score of wari

Wari	Color	Appearance	Taste	Texture	Flavor	Overall Acceptability
Fermented	8.6	8.3	8.7	8.6	8.8	8.6
Control	8.2	7.8	8.0	7.7	8.5	8.0

The scores for texture, flavor ,color, appearance and taste were observed to be high for fermented wari sample than control samples.

4.5.2 Graphical optimization of blend ratios

4.5.2.1 Graphical optimization of blend ratios for fermented samples

In order to show the effect of variables and to determine the blend ratios for best result, contour plots were drawn using software SURFER 6.0. The contour equations

were developed by keeping other variables at optimum value and are reported in Table B-1, B-3, B-5, B-7, B-9 and B-11 respectively in Appendix B.

The contours are shown in Fig. 4.1 (A,B,C) for various combinations of interactive terms at optimum point i.e. at various combinations of horse gram, black gram and black soybean. It is clear from the Fig.4.1 that WAI was continuously increasing with increase in black soybean and it was decreasing with an increase in horse gram and black gram.

In Fig. 4.2 (2A) shows a minimax region at the centre, called as saddle point which shows that there was no effect of increase or decrease of horse gram and black soybean on OAA. Fig. 4.2 (2C) (2B) shows OAA was continuously increasing with increase in black soybean but decreasing trend was observed with increase in horse gram.

In Fig. 4.3 (3A) shows that the protein content was minimum near about centre point ,here no effect on protein of both variables was observed. Protein increased with increasing level of (horse gram and black gram). Fig.4.3 (3B) it can be observed that protein content did not change with the black gram at centre point but it increased away from the centre point. Fig.(3C)shows the protein content increased with increase in black soybean. It slightly decreased with increase in horse gram.

In Fig 4.4 (4A) shows that the rehydration ratio increases by increasing black gram and minimum effect was observed at near central point. Fig.4.4 (4B) and (4C) showed that as black soybean increased , rehydration ratio was also increase. It was also observed that horse gram and black gram had min effect on rehydration ratio.

Fig. 4.5 (5.A) shows the effect of black gram on tannin content continuously increasing with the increases in the level of horse gram Fig.4.5 (5B) shows that as the effect of horse gram increases, tannin content also increases. while little amount of tannin content increases , in case of black soybean. Fig 4.5 (6C) shows the effect of tannin content deceased at centre point.

The contours are shown in Fig. 4.6 (A,B,C) for various combinations of interactive terms at optimum point i.e. at various combinations of horse gram, black

gram and black soybean. It is clear from the Fig that phytic acid was continuously decreasing with increase in black soybean while it was increasing with an include in black gram.

4.5.2.2 Graphical optimization of blend ratios for control samples

In order to show the effect of variables and to determine the blend ratio for best result, contour plots were drawn using software SURFER 6.0. The contour equations were developed by keeping other variables at optimum value and are reported in Table B-2, B-4, B-6, B-8, B-10 and B-12 respectively of Appendix B.

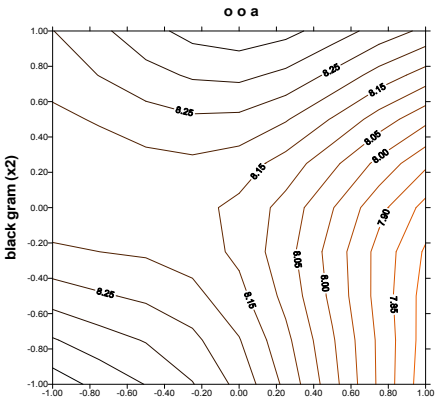
The contours are shown in Fig. 4.7 (A,B,C) for various combinations of horse gram, black gram and black soybean. Other variables was kept at optimum value for control samples. Fig. 4.7 shows a minimax region at the centre, called as saddle point. showing that there was no effect of increase or decrease of black gram and horse gram on rehydration ratio. Away from the saddle point, rehydration ratio increased with increase in black gram and decreased horse gram.

Fig. 4.8(8A) shows that the protein content was minimum at the central point. Away from the central point, protein content increased. Fig.8.(8C) Protein content continuously increased with an increase in black soybean because black soybean has highest amount of protein. Similar trend was observed in Fig. 4.8 (8B)

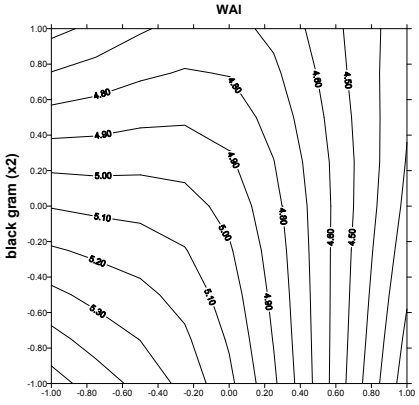
Fig. 4.9 (9A) and 9(C) shows that the tannin content decreased continuously with increase in black gram and increased with increase in horse gram. Fig.4.1(9B) showed that the tannin content increased with horse gram. A slight increase in protein was observed with increase in black gram.

Fig. 4.10 (10A) shows that phytic acid was increasing when the level of black gram and horse gram was increased. Fig. 4.10(C) shows that the phytic acid was minimum near about centre point. Fig 4.1 Fig. 4.10(10B) shows that the phytic acid decreased with an increase in black soybean.

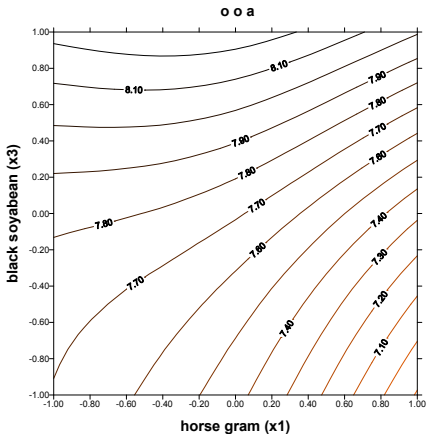
Fig.4.11(11 A) shows that black soybean and horse gram had a positive effect on water absorption index. Fig.4.11(11B) shows that a minimax region at centre point called as a saddle point. Which shows that there was no effect of increase and decrease



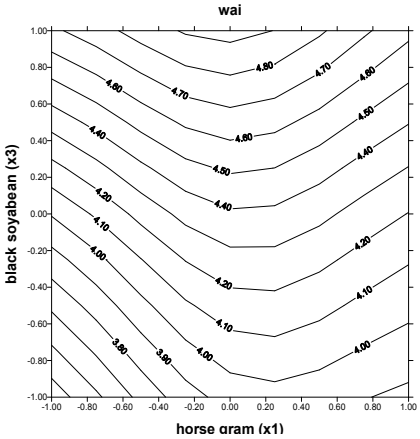
2A



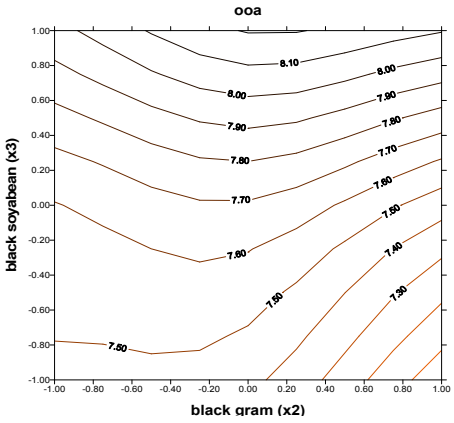
1A



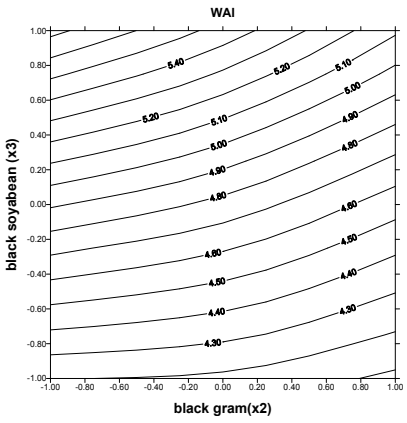
2B



1B



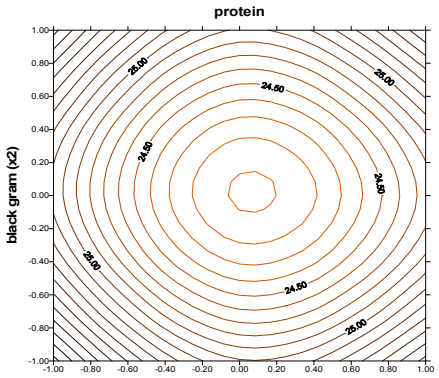
21C



1C

Fig 4.2: Contour plots for OAA for fermented samples

Fig 4.1: Contour plots for WAI for fermented samples



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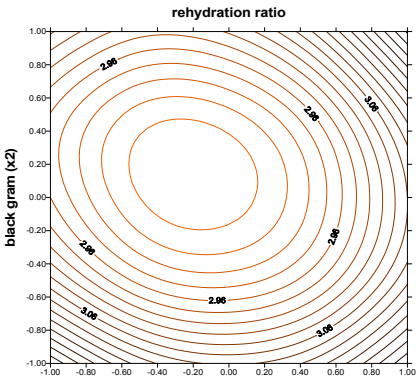

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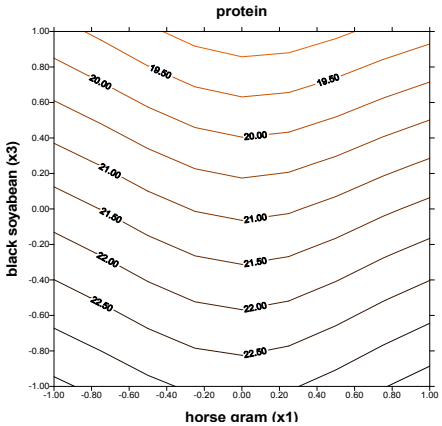

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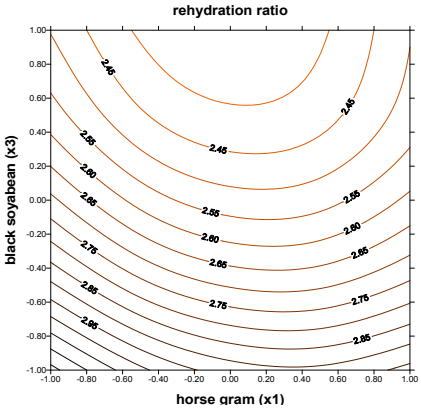

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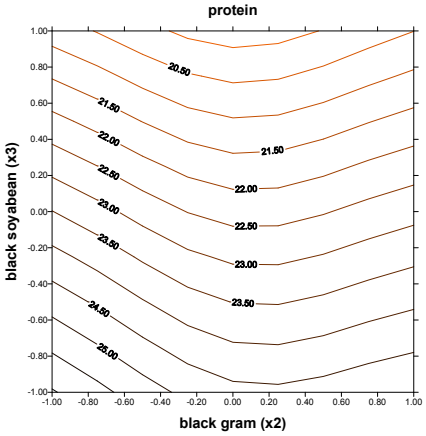

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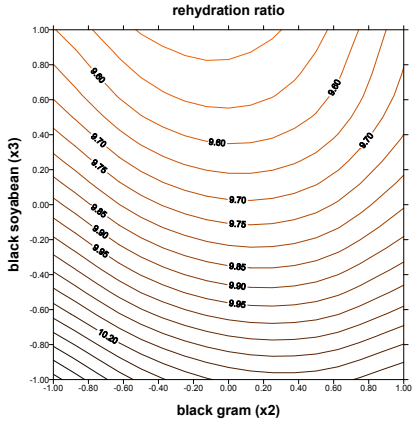
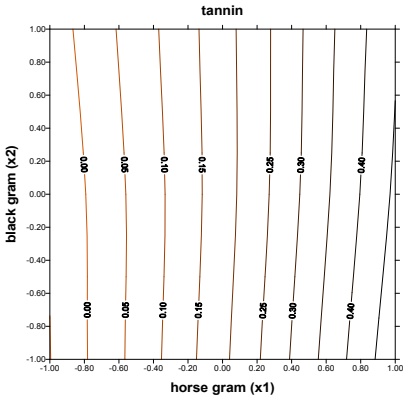


4C

Fig 4.3: Contour plots for Protein and samples

Fig.4.4: Rehydration ratio for fermented



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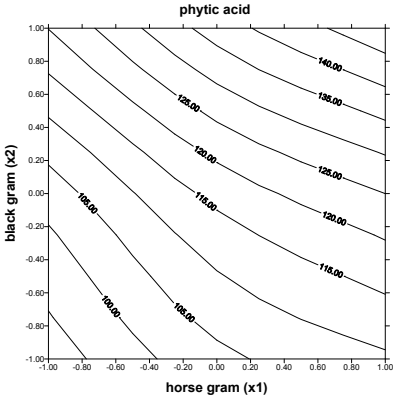

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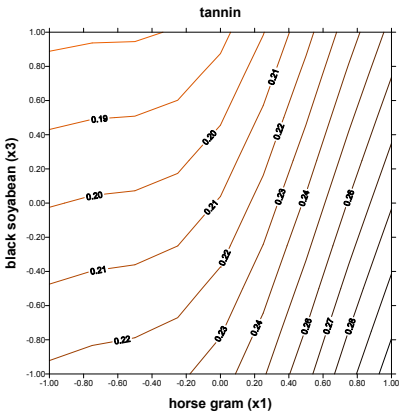

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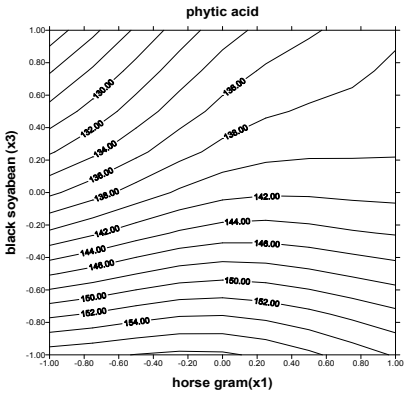

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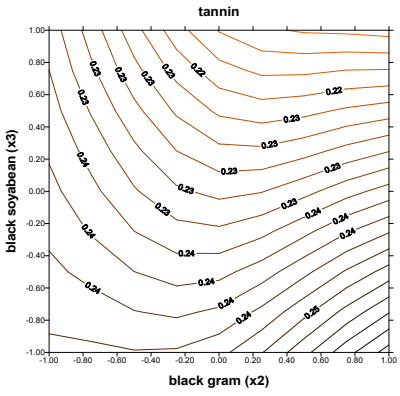

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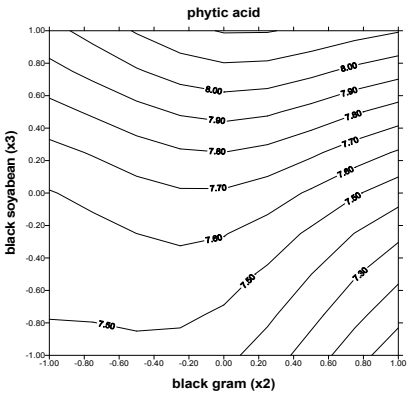
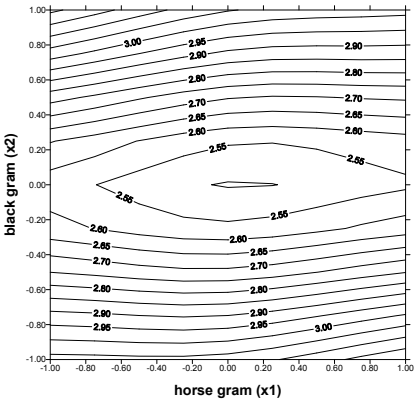


6C

Fig 4.5: Contour plots for Tannin and samples

Fig. 4.6: Phytic acid for fermented



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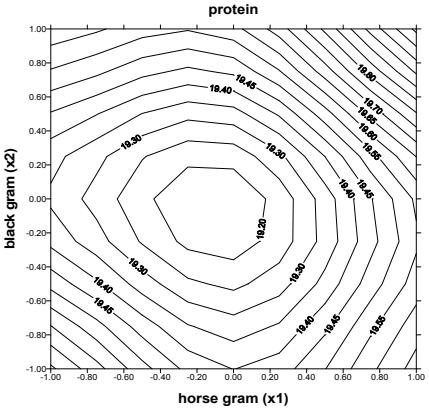

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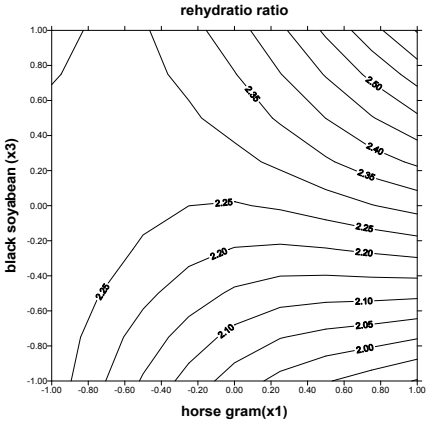

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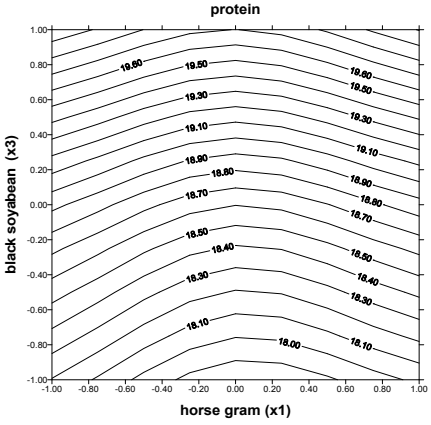

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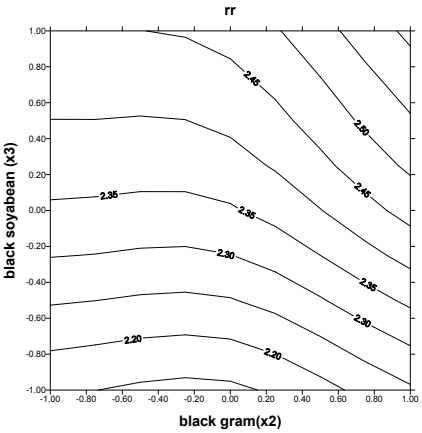

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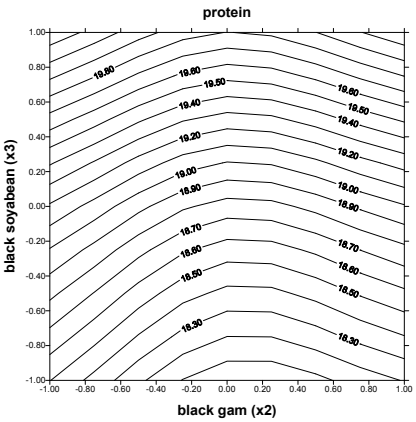
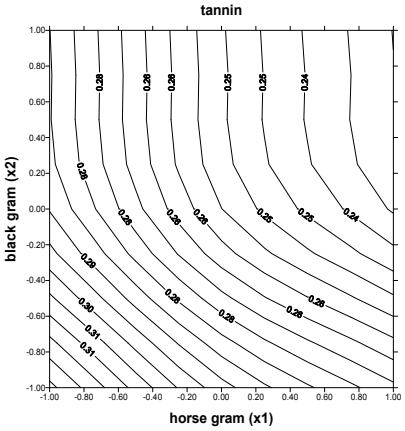


8C

Fig 4.7: Contour plots for Rehydration ratio and

Fig 4.8: Protein for control samples



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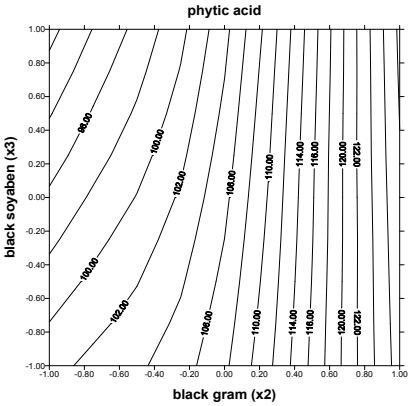

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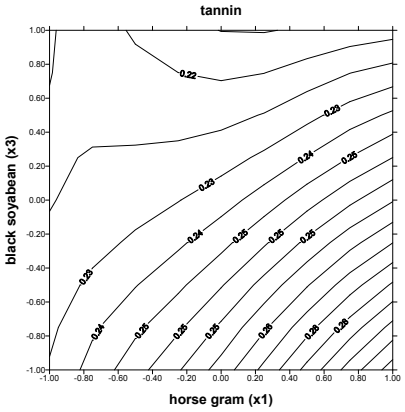

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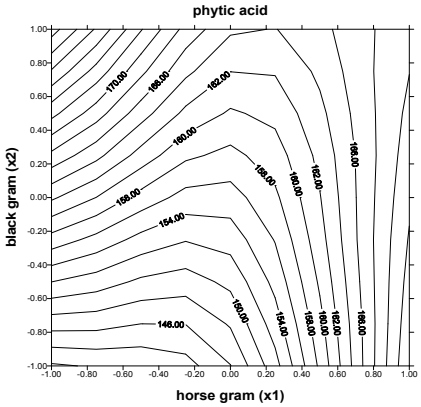

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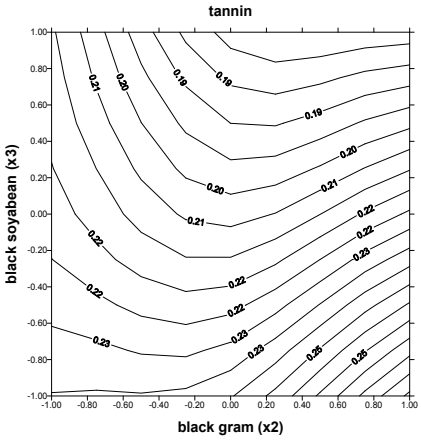

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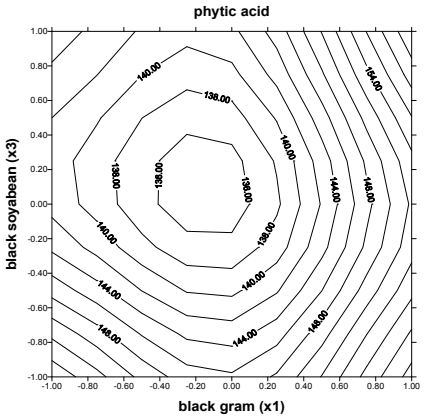
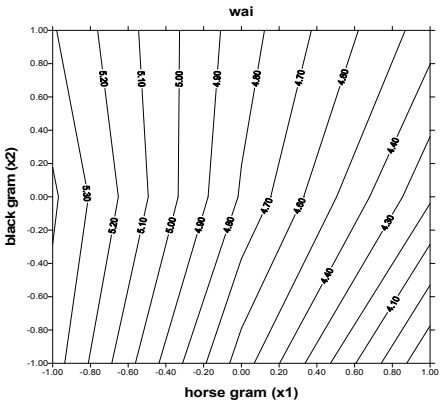


10C

Fig 4.9: Contour plots for Tannin and samples

Fig 4.10: Phytic acid for control



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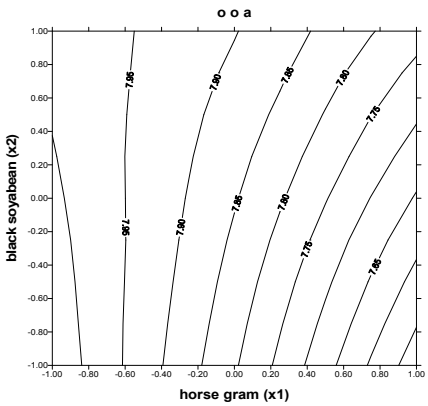

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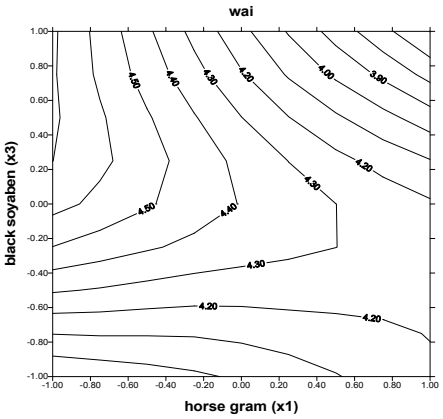

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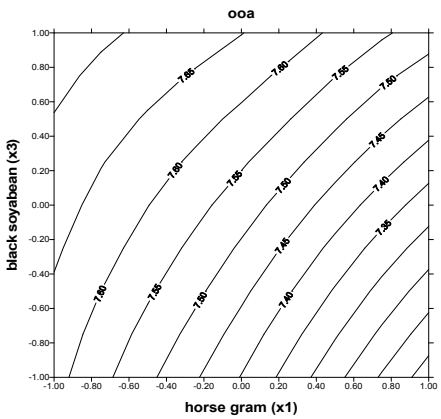

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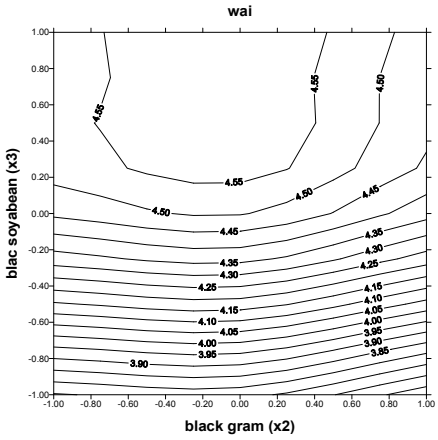

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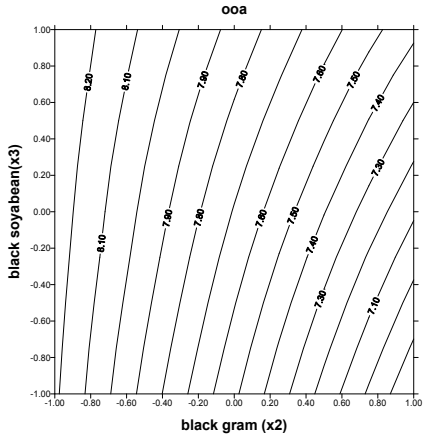


12C

Fig 4.11: Contour plots for WAI and samples

Fig. 1.12: Contour plots for Overall acceptability for control

of horse gram and black gram on water absorption index. Similar trend was observed in Fig 4.1(11C).

Fig. 4.12 (A,B,C) shows that the overall acceptability increases with increasing black gram and horse gram. Black soybean showed minimum effect on overall acceptability.

4.6 Overlapping of contours

4.6.1 Selection of variables

Various combinations of independent variables need to be selected as it is difficult to keep the variables exactly at the optimum point. This could be done by plotting contours of significant responses and overlaying one upon another. In present investigation, three responses viz., protein, phytic acid and rehydration ratio for which the model was found to be significant were selected for overlapping. The common area of four overlapping contours was used for range selection. For this, the minimum and maximum value of the responses was taken into consideration. Overlapping of contours was done on the basis of desirability of responses i.e. if response needs to be maximized, then difference of min and maximum values of response was taken and 25% of the difference was taken and then the value was added to the minimum value of response. The value thus obtained was considered as the selected point in contour lines. All the values falling below this point were deleted and remaining values were taken into consideration for drawing the contours. The range for responses selected for drawing the contours is reported in Table 4. Hence, all the points signifying the combination of variables, falling into the common area will give the broad range for responses considered.

Contour plots were developed by taking two independent variables into consideration at a time and keeping other independent parameters at the optimum point. Equations were used to develop contours given in Appendix-B. All the overlapped contours are shown in Fig. 4. Contour lines for protein, phytic acid and rehydration ratio are depicted by dark blue, sky blue and red color respectively. The common area of four contours has been highlighted by the shaded region. Any combination of the

response in the common area along with other responses at optimum point would give the better quality

Table 23: Value of responses for selecting range of variables from contour plots

Responses	Experimental values (actual)		For range selection	
	minimum	Maximum	minimum	Maximum
Protein	19.35	25.45	19.35	23.925
Phytic acid	104.73	149.62	115.95	149.62
Rehydration ratio	2.15	3.15	2.15	2.9

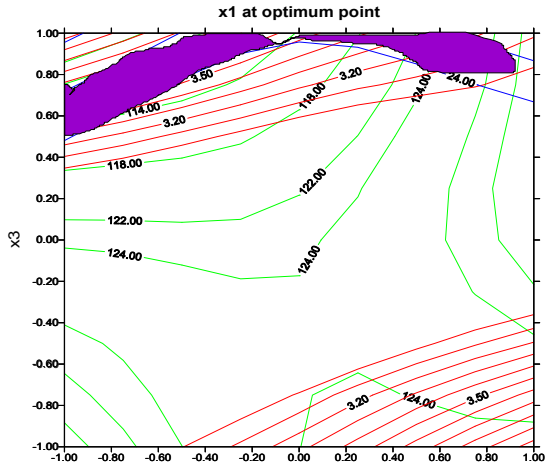
From the Fig.13 (A,B,C) it is very much evident that the optimized blend ratio range (Hg 63.5, Bg 50 and Bs 150) lie in the colored zones indicating the range for responses considered for optimization.

Table 4.24: Optimum values of blend ratios for fermented wari

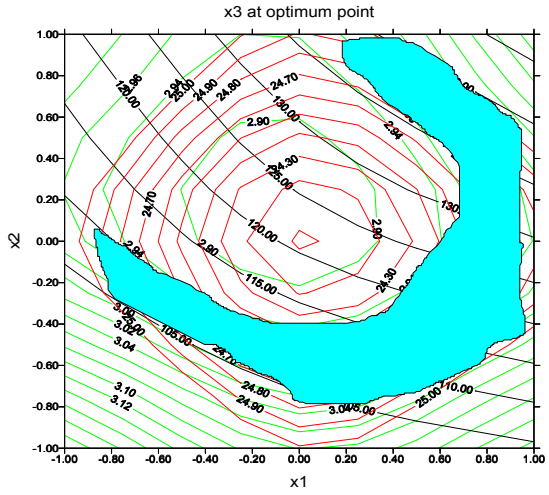
Value	Horse gram(g) (X_1)	Black gram(g) (X_2)	Black soybean (g) (X_3)
Coded	-0.73	-1	+1
Actual	63.5	50	150

4.7 Fermentation kinetics of wari batter

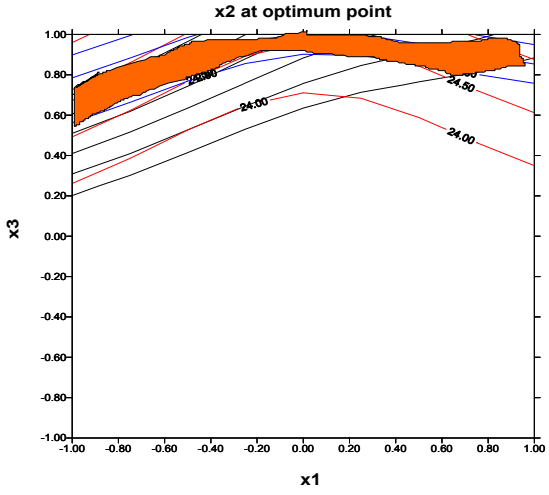
Fermentation kinetics of optimized blend ratio (obtained from Phase 1) was studied to see the growth patterns of micro organism involved during the process of fermentation. For this, fermented volume of batter was recorded (at an interval of 1 hr) against time. Experimental data is reported in Table no. 4.25 and this pattern is depicted in Fig. 14



(a)



(b)



(c)

Fig. 13 (A,B,C): Overlapped contour plots of x1,x2,x3 for optimum point.

It is observed from Fig.14 that is the growth pattern of micro organism followed the normal growth cycle i.e. have 4 stages of growth kinetics. These four phases are-lag phase, log phase (exponential), stationary phase and death phase.

In the Fig. 14 A-B denotes the lag phase which shows that there was no multiplication of micro organism, indicating no increase in cell number. B-C denotes the exponential phase (log phase), which ensures the growth of micro organism very fast. During this phase, the cells number increases exponentially with time and during this phase, maximum growth was observed. Table 25 also shows that the micro organism started increase in number after 2 hrs of fermentation. Initially, the increase was very much little in terms of growth but as the time of fermentation increased the growth was maximum after 9 hrs of fermentation.

It is evident from the Fig. 14 that the length of lag phase was very much less and it took less time to enter into the log phase. After the log phase the C-D denotes the stationary phase which shows by this time the micro organism attained the full growth and did not multiply. The micro organism remained as such. During D-E phase the micro organism started declining in numbers, which is called as death phase. During the fermentation kinetics, it suggested that fermentation should be carried up to the exponential phase only. i.e. A-C because the fermentation process beyond the exponential phase may result in producing toxins and odd flavor, that could be harmful for health.

It is also evident from the Table 4.25 that the fermented volume observed in case of wari batter was maximum after 9 hr. After this period the volume started decreasing and that decrease was due to the change in growth pattern of micro organism.

Table 4.26 shows the average time of fermentation and average volume of peak fermentation at room temperature. It clearly indicates that the total time of fermentation for fermented batter should be (10 hrs. at room temperature).During this period the maximum increase in fermented volume was observed.

Table 4.27 shows the different phases of microbial growth. The lag phase continued up to 2 hrs, the exponential phase of microbial growth continued up to 8 hrs

followed by stationary and death phase, indicating decrease in fermented volume of wari batter. The reason behind this could be due to the fact that during death phase the growth of micro organism declined and fermentation gasses scrapped, which resulted decrease in volume of wari batter.

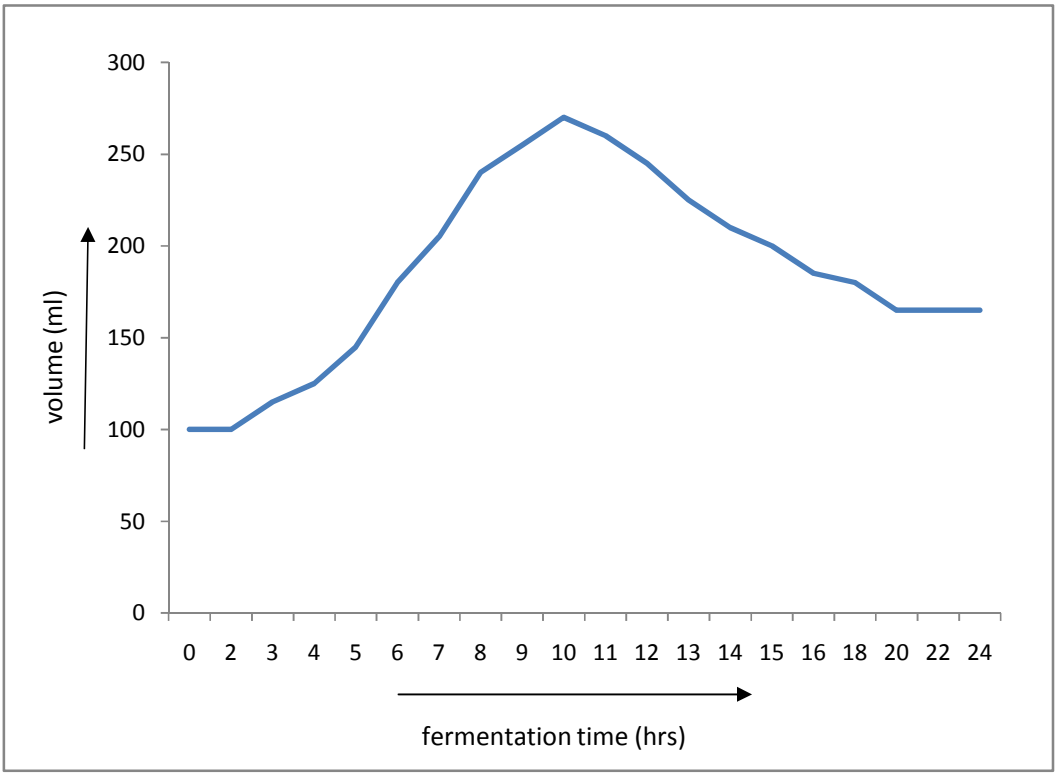


Fig. 14: Fermented volume of optimized wari batter at room temperature

Table 4.25: Average fermented volume of optimized wari batter at room temperature

Time of Fermentation(hr)	Temperature (ºc)	Fermented Volume (ml)
0	30 ºc	100
2	35ºc	100
3	35ºc	115
4	35ºc	125
5	35ºc	145
6	34ºc	180
7	33ºc	205
8	31ºc	240
9	30ºc	255
10	30ºc	270
11	30ºc	260
12	30ºc	245
13	29ºc	225
14	29ºc	210
15	28ºc	200
16	28ºc	185
18	27ºc	180
20	26ºc	165
22	28ºc	165
24	32ºc	165.

Table 4.26: Estimate average time of fermentation and average volume for maximum fermentation at room temperature.

Fermentation temperature (ºc)	Time of fermentation (hr)	Fermented volume(ml)
Room temperature.	10	270

Table 4.27 Time of fermentation of wari batter

Fermentation temperature (°c)	Time of fermentation (hr)		
Room temperature	Lag phase	Exponential	Total
	2	8	10

4.8 Fermentation kinetics studies

4.8.1 Kinetics models

Growth reaction kinetics of fermented wari batter was studied. Duration of each phase of fermentation was taken into consideration for the study. Kinetic models were tested to describe different transient growth situations. Since the maximum volume of wari batter was observed up to exponential phase, time of fermentation was considered up to that period only. (A-C only).

The fermentation kinetics represented by the changes in volume of wari batter was modeled in the form of growth kinetics of microorganisms, the cell growth pattern follows the equation as suggested by (Bailey and Ollis, 1986) during exponential growth phase.

$$X = X_0 e^{\mu t} \qquad (4.8)$$

Where,

X= volume of wari batter at any time t, ml

X_0 = initial volume of wari batter, ml

μ = Specific Fermentation Rate, 1/hr

t = time of fermentation, hr

4.8.2 Model Parameters Determination

In order to use the equation 4.8, the value of parameter can be estimated from fermentation kinetic data by graphical plotting of the above equation in its linearized form Fig 15 shows the linearized form of fermentation kinetics data.

Equation 4.8 can be linearized in the following form,

$$\ln(X/X_0) = \mu t \qquad (4.9)$$

So, if the plots of ln (X/X_0) vs. t, were to show linearity, the parameter μ could be determined from the slope. The data was plotted up to the time for maximum fermented volume, i.e. up to the apparent end of exponential phase of growth, Table 4.27.

4.8.3 Evaluation of Model for Fermentation Kinetics of WARI batter

The values of $\ln(X/X_0)$ and t as calculated from the volume vs. time data, Table 4.28 at room temperatures are tabulated in Table 4.25 , and are plotted in Fig.4.15 A linear trend was observed. Straight lines were fitted by performing linear regression and the parameter values were determined. The values of specific growth Rate (μ) so obtained at room temperature. are reported in Table 4.28. Growth data shows an acceptable fit of the proposed model in its linearized form to the experimental data in all cases.

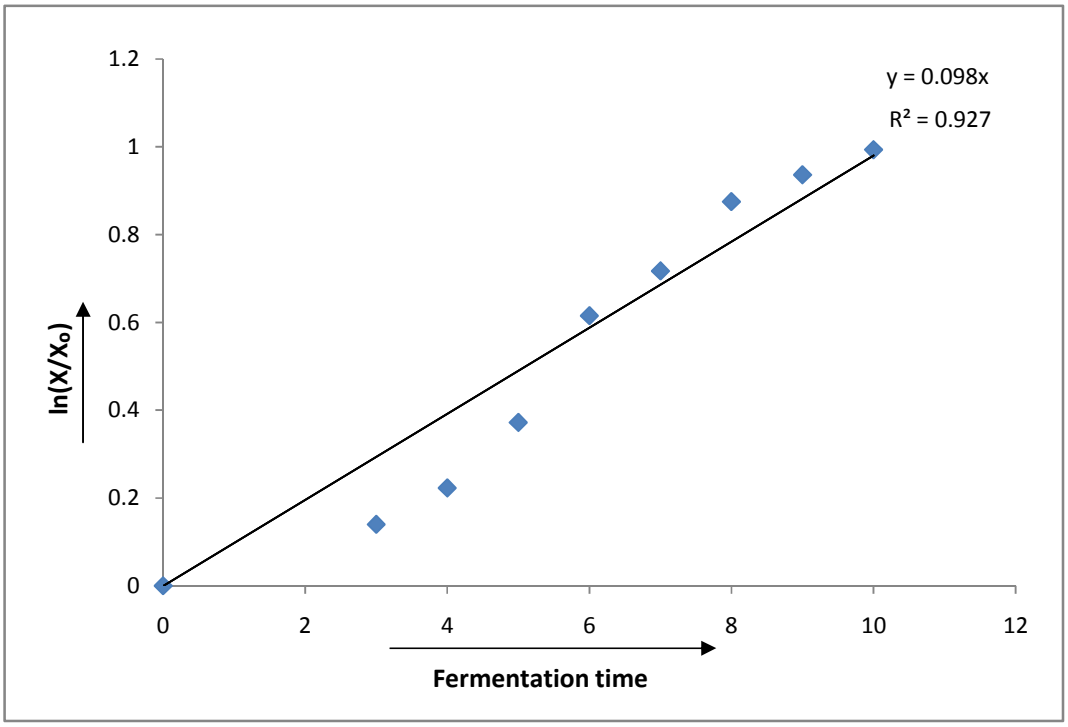


Fig. 17: Graphical plot of the kinetic model.

Table 4.28: Log of fermented volume ratio, ln (X/X_0) at room temperature

Time of fermentation, t	X	X_o	X/X_o	$ln(x/x_o)$	μ
0	100	100	1	0.00	0.00
2	100	100	1	0.00	0.00
3	115	100	1.15	0.14	0.047
4	125	100	1.25	0.22	0.055
5	145	100	1.45	0.37	0.074
6	180	100	1.80	0.66	0.11
7	205	100	2.05	0.72	0.103
8	240	100	2.40	0.88	0.11
9	255	100	2.55	0.94	0.104
10	270	100	2.70	0.99	0.099

Summary and Conclusion

Chapter 5 SUMMARY AND CONCLUSION

The present work was directed towards the development of a standardized process for horse gram and black soybean based wari using fermentation technology. Objectives were to investigate the effect of fermentation on quality of wari and to study the fermentation kinetics.

Experiments were designed using box benkhen design having 3 variables (horse gram, black gram and black soybean) and (3 levels of each). Design expert 7.0.0 was used for designing the experiments. Variables set for all the experiments were horse gram (50g, 100g, 150 g), black gram (50g, 100g, 150g) and black soybean (50g, 100g, 150g). Designed experiments were conducted to find the effect of these variables on water absorption index, protein, tannins, rehydration ratio, phytic acid, and overall acceptability. The designed experiments were performed in random manner and responses were determined for all the experiments. .

Data obtained from the statistical analysis was utilized to get the optimized blend ratios of all the independent variables (horse gram, black gram and black soybean). Numerical optimization was done after setting the constant values for the responses and the independent variables. Graphical optimization was done by drawing contours independent variables and responses. Overlapping of contours was done to decide the range of optimized variables.

Experimental studies revealed that fermentation process enhance the nutritional properties and decrease the anti-nutritional properties of selected grains. In all the cases it was observed that the fermented samples has higher nutritional content then control samples and lower anti-nutrients content then control samples. Fermented samples showed an increase in protein content (18.23%) then control samples. Decrease in tannin and phytic acid content was observed to be 12.12% and 24.08% respectively, while water absorption index, rehydration ratio, and overall acceptability showed an increase (9.71%, 15.9% and 3.5% respectively).

Results of the experimental study show that during fermentation, protein varied from 19.35 to 25.45 %. Range for water absorption index, rehydration ratio and overall

acceptability varied from 3.25-5.15 g/g, 2.25-3.15 and 7.1-8.5% respectively. The maximum and minimum values of anti-nutritional factors viz., tannins and phytic acid were found to be 0.285-0.165% and 149.62-104.73mg/100 g respectively.

The coefficient of determination (R^2) for the regression model of protein was 96.62%, for tannin it was 88.56%, for phytic acid 93.85%, for 92.08%, for rehydration ratio 93.04%, for water absorption index 94.34%, for overall acceptability 98.43% which indicates the significance of models. Of all the parameters considered for the study, Black soybean affected the protein at 1% level of significance while black gram and horse gram at 10% level of significance. Black gram affected the tannin content most at 5% level of significance and horse gram and black soybean did not affect the tannin content. Horse gram, black gran and black soybean affected the phytic acid at 1% level of significance. Black soybean affected the rehydration ratio at 1% level of significance while there was no effect of black gram and horse gram on rehydration ratio. Horse gram, black gram and black soybean affected the overall acceptability at 1% level of significance. Water absorption index was affected by horse gram and black soybean at 1% level of significance while black gram affected the water absorption index at 10% level of significant.

Through the experimental studies conducted under already described conditions followings conclusions could be drawn.

1. A process protocol for formulation of horse gram, black gram and black soybean based wari has been developed using fermentation technology. The fermented batter could give the wari with acceptable quality and high amount of protein and rehydration ratio. Anti-nutritional content (tannin and phytic acid) was also found to be decreased for fermented wari.
2. The fermentation kinetics studies reveal that the total length of fermentation for formulation of wari should be up to 10 hrs as temperature (30-35ºC). During this period maximum fermented volume was about 2.7 times of the original. Beyond this period the batter may develop bad aroma and odd flavours.
3. Microbial growth kinetics (all 4 phases) lag phase, log phase, stationary phase and death phase was observed during the study of fermentation kinetics. The kinetics showed that the rise in fermentation volume followed the normal growth cycle.

4. The model developed for wari batter during the fermentation kinetics (exponential phase) was $X=X_o\ e^{\mu t}$
5. The specific growth rate was observed to be $\mu=0.099$ and R value for the develop model was 92.7.

Hence, it could be recommended that for developing acceptable quality of (horse gram, black gram and black soybean) based wari, the best suitable fermented blend ratio should be (63.5g: 50g: 150g). The maximum expansion (2.7 times of the original batter) in batter volume could be obtained at 30-35ºC using 10 hrs of fermentation.

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Appendices

APPENDIX A

Table A.1Preparation of standard curve for estimation tannin content

Std. solution (ml)	Absorbance	mg of tannin
0	0	0
5	0.046	0.5
6	0.021	0.6
7	0.048	0.7
8	0.074	0.8
9	0.084	0.9
10	0.092	1

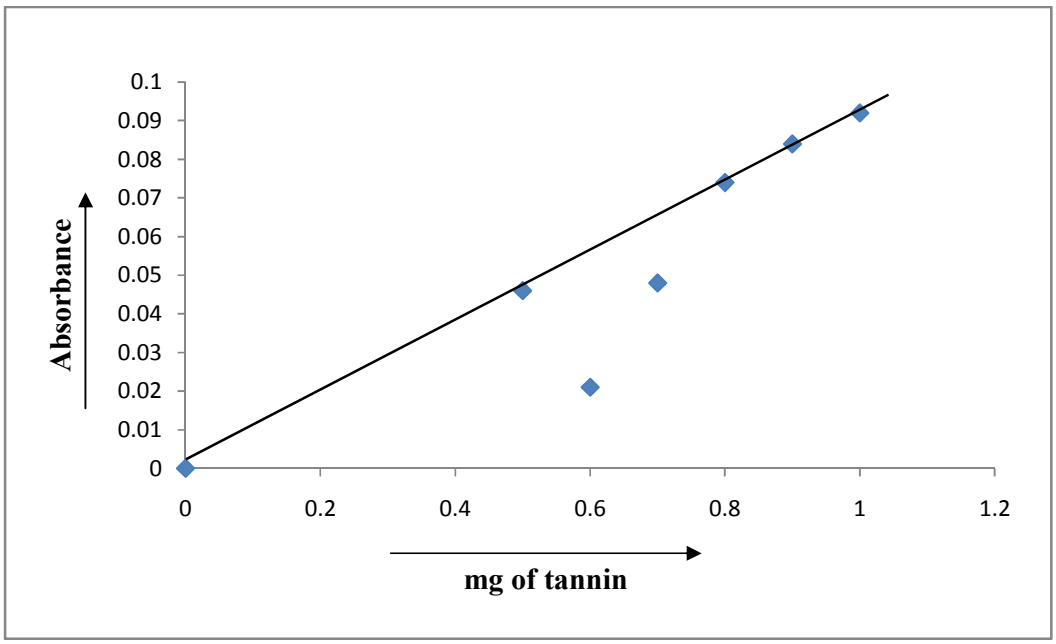

Fig A_1 Standard curve for tannin

Table A.2 Preparation of standard curve for estimation phytic acid content

Std. solution (10 mg/100 ml)	Absorbance
10	0.06
20	0.14
30	0.22
40	0.32
50	0.42
60	0.48
70	0.56
80	0.62
90	0.68
100	0.72

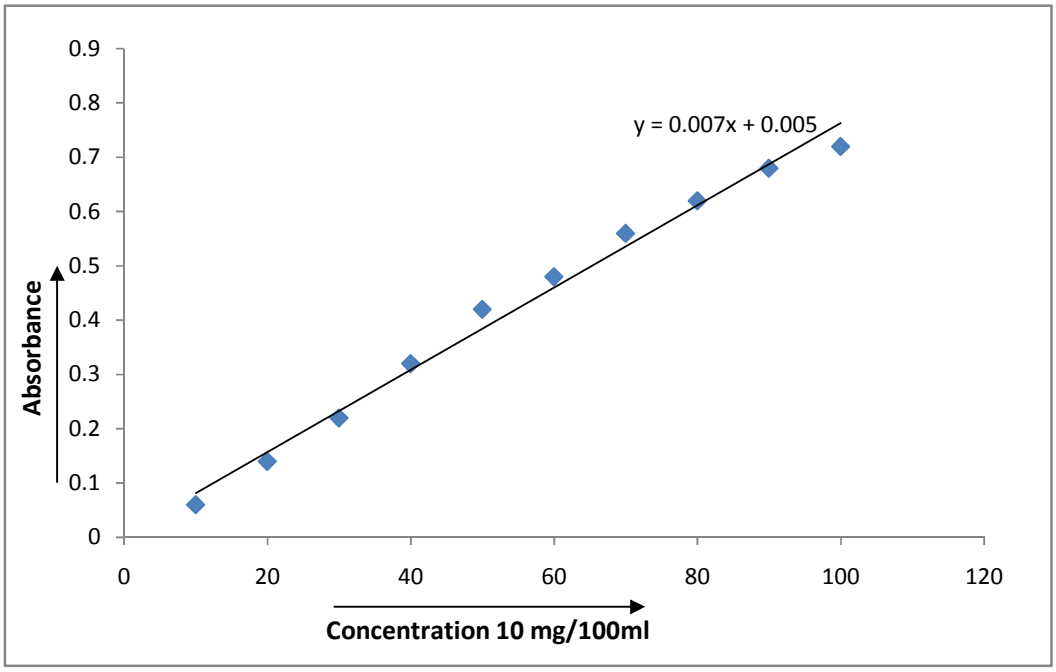

Fig A_2 Standard curve for phytic acid

Table A.3 Preparation of standard curve for estimation protein content

Sr. no.	Std./sample (µg/tube)	Amount(µl)	Water(µl)	Absorbance (A_{595} nm)
1	0	0	100	0
2	10	10	90	0.084
3	20	20	80	0.096
4	40	40	60	0.224
5	60	60	40	0.352
6	80	80	20	0.486
7	100	100	0	0.626

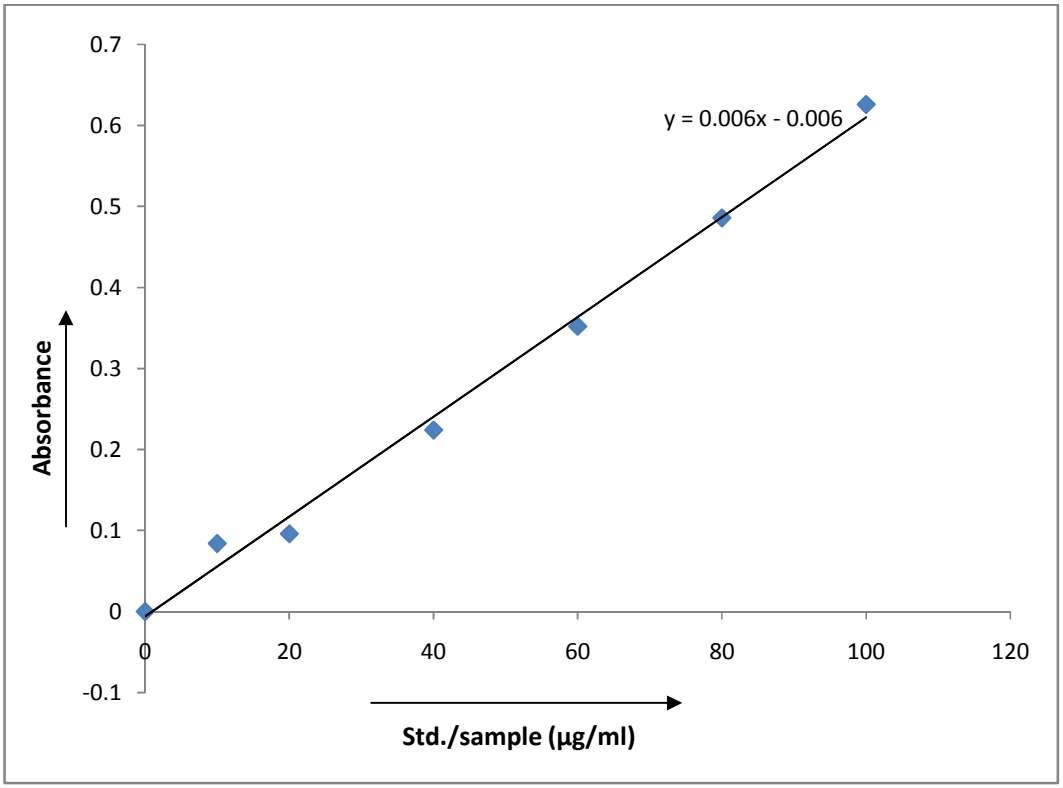

Fig A_3 Standard curve for protein

Table B.1 Contour equation for protein at optimum point for fermented samples

XY Parameter	Variable		Contour equations
X_1X_2	X_3	+1	$z=24.08+0.03X_1+0.15X_2+0.05X_1X_2+1.02X_1^2+1.07X_2^2$
X_1X_3	X_2	-1	$z=22.78+0.13X_1+2.35X_3-0.15X_1X_3+1.02X_1-0.13X_3^2$
X_2X_3	X_1	-0.73	$z=22.01-0.13X_1+2.71X_2+0.25X_1X_2+1.07X_1^2-0.13X_2^2$

Table B.2 Contour equation for protein at optimum point for control samples

XY Parameter	Variable		Contour equations
X_1X_2	X_3	1	$z=19.92+0.21X_1+0.20X_2+0.16X_1X_2+0.36X_1^2+0.41X_2^2$
X_1X_3	X_2	-0.42	$z=18.47+0.04X_1+1.1X_3+0.10X_1X_3+0.36X_1^2+0.19X_3^2$
X_2X_3	X_1	-1	$Z=18.85-0.02X_2+1.03X_3+0.063X_2X_3+0.41X_2^2+0.19X_3^2$

Table B.3 Contour equation for tannin at optimum point for fermented samples

XY Parameter	Variable		Contour equations
X_1X_2	X_3	+1	$z=0.17+0.028X_1-0.005X_2-0.02X_1X_2+0.035X_1^2+0.01X_2^2$
X_1X_3	X_2	-1	$z=0.21+0.05X_1-0.03X_3-0.003X_1X_3+0.04X_1^2+ 0.0003X_3^2$
X_2X_3	X_1	-0.73	$z=0.19-0.0004X_2-0.014X_3-+0.01X_2X_3+0.01X_2^2+ 0.0003X_3^2$

Table B.4 Contour equation for tannin at optimum point for control samples

XY Parameter	Variable		Contour equations
X_1X_2	X_3	1	$z=0.21+0.03X_1-0.02X_2+0.00X_1X_2+0.01X_1^2 +0.02X_2^2$
X_1X_3	X_2	-0.41	$z=0.23+0.02X+0.01X_3+0.01X_1X_3-0.01X_1^2+0.004X_3^2$
X_2X_3	X_1	-1	$z=0.22+0.003X_2-0.03X_3- 0.02X_2X_3+0.02X_2^2 +0.004X_3^2$

Table B.5 Contour equation for phytic acid at optimum point for fermented samples

XY Parameter	Variable		Contour equations
X_1X_2	X_3	+1	$z=122.4 +12.27X_1+ 20.03X_2+3.63X_1X_2-3.54X_1^20-4.85X_2^2$
X_1X_3	X_2	-1	$z=122.29 +3.05X_1- 12.37X_3 + 5.59X_1X_3 -3.54X_1^2 -5.4X_3^2$
X_2X_3	X_1	-0.73	$z=123.47+ 6.37X_1- 5.44X_2+11.01X_1X_2+ 4.85X_1^2-5.4X_2^2$

Table B.6 Contour equation for phytic acid at optimum point for control samples

XY Parameter	Variable		Contour equations
X_1X_2	X_3	1	$z=126.53 +6.68X_1+8.81X_2-14.1X_1X_2+ 14.63X_1^2+ 8.06X_2^2$
X_1X_3	X_2	-0.41	$z=135 + 9.37X_1-0.28X_3+3.15X_1X_3+14.63X_1^2-11.04X_3^2$
X_2X_3	X_1	-1	$z=147.41+19.39X_2 -1.99X_3+ 3.52X_2X_3 +8.056X_2^2-11.04X_3^2$

Table B.7 Contour equation for rehydration ratio at optimum point for fermented samples

XY Parameter	Variable		Contour equations
X_1X_2	X_3	+1	$z=2.87+0.05X_1-0.037X_2+0.05X_1X_2+0.14X_1^2+0.17X_2^2$
X_1X_3	X_2	-1	$z=2.52-0.05X_1-0.44X_3+0.05X_1X_3+0.014X_1^2+0.12X_3^2$
X_2X_3	X_1	-0.73	$z=2.46+0.002X_2+0.32X_3-0.08X_2X_3+0.17X_2^2+0.12X_3^2$

Table B.8 Contour equation for rehydration ratio at optimum point for control samples

XY Parameter	Variable		Contour equations
X_1X_2	X_3	1	$z=2.4-0.01X_1+0.01X_2-0.1X_1X_2+0.08X_1^2+0.075X_2^2$
X_1X_3	X_2	-0.41	$z=2.29+0.05X_1+0.21X_3-0.03X_1X_3+0.08X_1^2-0.05X_3^2$
X_2X_3	X_1	-1	$z=2.31+0.09X_2+0.18X_3+0.03X_2X_3+0.08X_2^2-0.05X_3^2$

Table B.9 Contour equation for WAI at optimum point for fermented samples

XY Parameter	Variable		Contour equations
X_1X_2	X_3	+1	$z=4.91-0.49X_1-0.02X_2+0.36X_1X_2-0.35X_1^2+0.05X_2^2$
X_1X_3	X_2	-1	$z=4.39-0.07X_1+0.65X_3-0.15X_1X_3-0.35X_1^2-0.07X_3^2$
X_2X_3	X_1	-0.73	$)4-0.23X_2+0.71X_3-0.05X_2X_3-0.05X_2^2-0.07X_3^2$

Table B.10 Contour equation for WAI at optimum point for control samples

XY Parameter	Variable		Contour equations
X_1X_2	X_3	1	$z=3.84-0.64X_1+0.17X_2+0.21X_1X_2+0.036X_1^2-0.11X_2^2$
X_1X_3	X_2	-0.41	$z=4.2-0.296X_1-0.06X_3-0.43X_1X_3+0.04X_1^2+0.4X_3^2$
X_2X_3	X_1	-1	$z=4.54-0.07X_2+0.38X_3+0.03X_2X_3-0.11X_2^2-0.4X_3^2$

Table B.11 Contour equation for Overall acceptability at optimum point for fermented samples

XY Parameter	Variable		Contour equations
X_1X_2	X_3	+1	$z=8.13-0.2X_1-0.14X_2+0.20X_1X_2-0.17X_1^2+0.21X_2^2$
X_1X_3	X_2	-1	$z=7.73-0.10X_1+0.59X_3-0.10X_1X_3-0.17X_1^2+0.16X_3^2$
X_2X_3	X_1	-0.73	$z=7.67-0.11X_2+0.48X_3-0.18X_2X_3+0.21X_2^2+0.16X_3^2$

Table B.12 Contour equation for Overall acceptability at optimum point for control samples

XY Parameter	Variable		Contour equations
X_1X_2	X_3	1	$z=7.84-0.20X_1-0.63X_2-0.1X_1X_2-0.03X_1^2-0.01X_2^2$
X_1X_3	X_2	-0.42	$z=7.52-0.19X_1+0.14X_3-0.05X_1X_3-0.03X_1^2+0.14X_3^2$
X_2X_3	X_1	-1	$z=7.72-0.06X_1+0.21X_3+0.03X_2X_3-0.01X_2^2+0.14X_3^2$

APPENDIX C

EVALUATION CARD FOR HEDONIC RATING TEST

Name of Tester: Date:

Product: **Rehydrated fermented wari sample** Time:

- Test this sample and check appropriate blank how much you like or dislike.
- Use the appropriate scale to show your attribute by checking at the point that best describes your feeling about the sample.
- Please give reason for this attribute.
- Remember you are the only who can tell what you like.
- An honest expression of your personal feeling will help us.

Scale:

Like extremely	9	Like slightly	6	Dislike moderately	3
Like very much	8	Neither like nor dislike	5	Dislike very much	2
Like moderately	7	Dislike slightly	4	Dislike extremely	1

VITA

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ABSTRACT

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Advisor : Dr. Anupama Singh

Diversification of food using underutilized crops must be encouraged both at national and household level. These underutilized crops contain relatively high protein content and also have some anti-nutritional content i.e. phytic acid, tannin. Fermentation technology could be one of the best methods for processing of these crops at house hold level.

Experiments were conducted to standardize the process for development of wari using blends of horse gram, black gram and black soybean. Product was formulated using fermentation technology and blend ratios were optimized. Fermentation kinetics for the optimized blend ratio was also studied in respect of the change in fermented volume with time and kinetics model was developed.

Box-Benkhen design of response surface methodology was used to design the experiments. The variables selected for the final experiments were three levels of horse gram(50g,100g,150g), black gram(50g,100g,150g), and black soybean (50g,100g,150g). Designed experiments were conducted randomly to find the effect of these variables on protein, tannin, phytic acid, rehydration ratio, water absorption index and overall acceptability. The data from all experiments were analyzed statistically using Design Expert 7.0.0 and the response functions were developed using multiple regression analysis and second order models were fitted for each response. Using the regression equations of the variables, contours were drawn with SURFER 6.0.

All the fermented and control samples were subjected to proximate analysis, functional and anti-nutritional properties. Optimized blend ratios having high value of protein, rehydration ratio, water absorption index and low values of tannin, phytic acid was found to be as 63.5(Hg):50(Bg):150(Bs). This optimized blend ratio was selected for the fermentation kinetics studies. The effect of fermentation was found to be very effective as protein increased by 18.23 % and anti-nutritional factors, tannin and phytic acid were found to be reduced by 12.12 % and 24.8%. Fermentation process played an important role as rehydration ratio, water absorption index and overall acceptability was higher (15.87%, 9.7% and 3.5% respectably) for fermented wari samples.

Optimization of blend ratios was done with a view to enhance the protein level, water absorption index and minimize the phytic acid, tannin content in a developed product (wari). The model F- value was found to be highly significant at 1% level of significance in case of all the responses. Hence, second order model could be fitted to predict all the dependent parameters. Study of fermentation kinetics was also done and the specific growth rate (μ) and regression coefficient (R) value were reported to be 0.099 and 92.7 respectively. 10 hrs of fermentation was observed to be appropriate as no growth was observed beyond this time. The range of μ and R shows an acceptable fit of the proposed model in its linearized form to the experimental data in all cases.

(Anupama Singh)
Advisor

(Ankita Roy)
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मुख्य विषय	:	खाद्य जैव तकनीकी अभियंत्रिकी	विभाग	:	कटाई उपरान्त प्रसंस्करण खाद्य अभियंत्रिकी
शोध शीर्षक	:	"गहद एवं भट्ट पर आधारित वरी के अनूकूलित मिश्रण अनुपात के लिए किण्वन कैनेटीक्स अध्ययन"			
सलाहकार	:	डा0 अनुपमा सिंह			

सारांश

न्यूनउपयोजित फसलों का उपयोग करके राष्टीय एवं घरेलू दोनों स्तर पर खाद्य उत्पादों के विविधीकरण को प्रोत्साहित किया जाना चाहिए। इन फसलों में उच्च प्रोटीन अव्यय के साथ पोषकरोधी तत्व जैसे फाइटिक अम्ल, टैनिन भी उपस्थित होते हैं। घरेलू स्तर पर अनाजों के प्रसंस्करण के लिए 'किण्वन' तकनिकी उत्तम विधियों में से एक है।

वरी बनाने की प्रक्रिया को और उन्नत बनाने के लिए मिश्रण अनुपात भट्ट, गहद एवं उर्द का उपयोग किया गया है। इस उत्पाद को बनाने के लिए किण्वन तकनीकी का उपयोग करके मिश्रित अनुपात को इष्टतम किया गया।

इष्टतम मिश्रित अनुपात के लिए किण्वन गतिकी का अध्ययन, समय के साथ किण्वन आयतन में आए अन्तर को देखकर किया गया था। पद्वति प्रयोगों को अभिकल्पित करने हेतु रिसपान्स सरफेस कार्यप्रणाली की बॉक्स बेंकन डिजाइन को उपयोग में लाया गया है। अन्तिम प्रयोगों के लिए भट्ट 50, 100, 150 ग्राम गहद 50, 100, 150 ग्राम एवं उर्द 50, 100, 150 ग्राम चरों का चयन किया गया है।

इन चरों का प्रोटीन, टैनिन, फाइलिक अम्ल, जलअवशोषित सूचनांक, समुचय स्वीकार्यता एवं पुनजलीयकरण अनुपात पर प्रभाव पता करने हेतु अभिकल्प विशेषज्ञ की सहायता से प्राप्त ऑकड़ो का सांख्यिक रूप से विश्लेषण किया गया तथा बहुगुणित समाश्रय विश्लेषण के प्रयोग से अनुक्रिया फलन विकसित किए गये एवं प्रत्येक अनुक्रिया के फलन विकसित किये गये एवं प्रत्येक अनुक्रिया के लिए द्वितीय कोटि प्रतिरूप आंसजित किये गये। चरों के समाश्रयण समीकरण के उपयोग द्वारा र्सफर 6.0 की सहायता से कंटूर चित्रित किए गये।

सभी किण्वन एवं नियंत्रित नमूनों को कार्यात्मक पोषक एवं पोषकरोधी परीक्षण में रखा गया। किण्वन गतिकी अध्ययन के लिए इष्टतम मिश्रण अनुपात गहद 65.5 ग्राम; उर्द 50 ग्राम; भट्ट 150 ग्राम को चुना गया। प्रोटीन, जलअवशोषित सूचनांक, समुचय स्वीकार्यता एवं पुनजलीयकरण अनुपात इष्टतम मिश्रित निम्न मात्रा में पाए गये। प्रोटीन में 18.23 प्रतिशत की वृद्वि तथा पोषकरोधी तत्व जैसे टैनिन, फाइलिक अम्ल में 12.12 प्रतिशत तथा 24.80 प्रतिशत की कमी पाई जाने के कारण किण्वन के प्रभाव को अत्यन्त प्रभावकारी पाया गया। जबकि पुनजलीयकरण अनुपात, जलअवशोषित सूचनांक एवं समुचय स्वीकार्यता 15.87 प्रतिशत 9.71 एवं 3.5 प्रतिशत मशः वृद्वि पाई गयी।

गहद, भट्ट एवं उर्द के लिए पोषकरोधी कारकों में अधिकतम कमी के लिए तथा प्रोटीन की अधिकतम वृद्वि के लिए इष्टतम परिस्थितियों को सांख्यिक विश्लेषण द्वारा प्राप्त किया गया। सभी माप–दण्डों के लिए प्रतिरूप एफ.मान 1 प्रतिशत सार्थकता स्तर पर अधिक सार्थक पाए गये। अतः सभी निर्भर मापदण्डों के पूर्वानुमान के लिए द्वितीय कोटि प्रतिरूप आंसजित किए जा सकते हैं।

किण्वन गतिकी का अध्ययन भी किया गया एवं विशिष्ट वृद्वि दर μ तथा प्रतिगमन गुणांक R^2 मशः 0.099 तथा 0.927 मान को भी दर्शाया गया है। 10 घंटे के किण्वन को उत्तम पाया गया है क्योंकि इस समय के उपरान्त सूक्ष्म जीवों में कोई वृद्वि नहीं देखी गई। μ एवं R की रेंज यह दर्शाता है कि प्रस्तावित निदर्श यदि अपने रैखिक रूप में है तो यह प्रयोगात्मक ऑकड़ो के लिए स्वीकृत है।

(अनुपमा सिंह)
सलाहकार

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