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Physiochemical Analysis of Bread Fortified with different Levels of Soyaflour Blends

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ABSTRACT

The present study carried out for bread fortification with different concentration of soybean flour. The main objective of this experimental study is to develop value added bread with improved nutritional value by fortification with different concentrations of soya blends. Fortification can be done up to 10% without affecting consumer acceptable limits of bread. Composite bread with soybean flour substitution were found to be nutritionally rich as the addition of soya flour to the whole wheat flour for bread preparation increase the protein and fat content of the bread. These breads are nutritionally superior as the level of lysine content increased due to increased protein content. As the moisture content increases with increase in soya concentration the risk to microbial spoilage hence shelf stability also increases. Therefore whole wheat bread was considered more suitable than soya substituted breads. Consumption of soy protein has been associated with benefits related to numerous areas of health. Different concentrations of SF (soya flour) were added to the whole wheat flour during bread manufacture. 5% 10% 15% 20% concentration of SF (soya flour) was used and different raw material testing was conducted such as protein estimation, ash content, moisture content, fat content, and SDS sedimentation values. The developed bread was tested using consumers for acceptability, using bread without fortification with SF as a control. The bread with least SF concentration was more acceptable and the texture was good and similar to control. As the concentration of SF increases the acceptability decreases as the beany flavor became more pronounced and the texture also was affected. The bread with more SF blends was stickier with higher moisture content and this lead to less acceptance of bread with 20% SF concentration by the consumers than control and bread upto 10% SF concentration.

Keywords: *Fortification,soya flour(SF),Protein concentration,Sensory Evaluation*

INTRODUCTION

Protein energy malnutrition or protein calorie malnutrition refers to a form of malnutrition where there is inadequate protein intake. PEM continues to be a major health burden in developing countries and most important risk factor for illness and death especially among children. It affects children the most because they have less protein intake .This is also secondary to other conditions such as chronic renal disease or cancer cachexia. The most recent estimates show that about 60% of all deaths, occurring among children aged less than 5 years in developing countries could be attributed to PEM. The health consequence most pronounced in children suffering from PEM includes higher susceptibility to infectious and metabolic disease. PEM is also associated with number of comorbidities such a lower respiratory tract infection including tuberculosis, diarrhea and anemia. Fortification of food by indispensible amino acids, supplementation with quality protein, improvement of protein quality by plant breeding and minimizing the damage to the nutritional value of protein caused during processing and packaging[18].

Food fortification or enrichment is process in which food product modified with the nutrients which were not originally present in the food product. The first fortification took place in 1821 when a French chemist jean added iodine to salt which help in reduction of goitre. In the rapidly changing scenario more highly processed foods has been used to justify the addition of nutrients to an expanding range of food in order to ensure nutritional adequacy of the diet. This has become an effective strategy to overcome micronutrient deficiency. As defined by world health organization and food and agricultural organization of united states (WHO and FAO, 1994) fortification refers to "the practice of deliberately increasing the content of essential micronutrients, that is vitamins, proteins and minerals in food irrespective of whether the nutrient were originally present in food before processing or not, so as to improve nutritional quality of food supply and to provide a public purpose of addition. Food fortification can provide relatively rapid solution to address low micronutrients intakes at population level whilst maintaining traditional dietary patterns[40]. The food vehicles should be carefully selected according to food habits of the population. The most common used food vehicle is cereals. Fortification of whole wheat bread with soya blend is an effective method for enrichment with proteins and can act as tool against protein energy malnutrition.

Wheat is unique among cereals for its ability to dough which help to process in bread and other baked products like cakes, biscuits, pasta and a range of processed food. The properties are designed from structure and interaction of grain storage protein which together form gluten fraction[42]. Wheat proteins are comprised of albumin 15% globulin 5% gliadin 33%, soluble glutenin 14% insoluble glutenin 33%. Glutens are made by 2 fractions of glutenin and Gliadins[42]. Gliadins are monomeric and interact by hydrogen bonding and hydrophobic interactions. These compositions of wheat are its properties of dough making and are major component used in bakery products. And bread is one of the most common bakery product formed from the cereal wheat.

Bread is one of the most staple diets. Combinations of different flours, and differing proportions of ingredients, has resulted in the wide variety of types, shapes, sizes, and textures available around the world. It may be leavened (aerated) by a number of different processes ranging from the use of naturally occurring microbes to high-pressure artificial aeration during preparation and/or baking, or may be left unleavened. The use of white flour derived from the processing of whole wheat grain, which is aimed at improving aesthetic value of white bread, has also lead drastic reduction in nutritional density and fiber content when bread compare to bread made from whole grain cereals[27]. Bread being good source of nutrient is deficient in proteins and certain essential amino acids. Bread being made from wheat can be used as vehicle for fortification and considered a good source of functional foods. Recently consumers' awareness to eat healthy and high quality food known as fortified or functional foods is increasing[32]. Functional foods have had a large impact on the role of diet and health. Food components that were once thought to be non-nutritive have since been found to be a key in disease prevention and overall maintenance of good health. In the United States and in many other countries there are many definitions for functional foods. However, there is an agreement that the term indicates foods that have a positive effect on health beyond normal nutrition when consumed. Many descriptions of functional food have been created by various organizations. The American Dietetic Association describes functional foods as foods that "have a potentially beneficial effect on health when consumed as part of a varied diet on a regular basis, at effective levels"[3]. A functional food is typically a food product that health claims may be made about to increase consumers' interest and purchases. In the United States a health claim is a claim on a food label that shows a relationship between a nutrient in the food and a disease or health-related condition[11]. The nutrient may be naturally found in a food, added to a food product from another food, added to a food product from somewhere other than the food supply, or added to a food product through the creation of a totally new food. Food processing technologies, traditional breeding, or genetic engineering may be used for these purposes. However, some of these methods may reduce the functionality of the nutrient and this should be taken into consideration. The enrichment of bread and other cereal based product with legumes flour particularly in region where protein utilization is inadequate has long been recognized. This is because legumes, nutritionally protein are high in mineral, vitamin B and lysine an essential limiting amino acid in most cereals.

Legumes therefore complement cereal when blended at optimum ratio. Legumes have a very specific place from the nutritive point of view and play an important role in nourishment of world population. Protein content in legume grains range from 17 % - 40 %, contrasting with 7 – 13 % of cereals[8], and being equal to the protein contents of meats that is 18 – 25 %. Addition of legumes to cereal products increases their fiber content resistant starch[47], important minerals[13] and vitamins. Their consumption has positive impact on health conditions of consumers[19,36]. Soya bean (*Glycine max*) is a species of legume native to East Asia, widely grown for its edible bean which has numerous uses. Soya bean are considered by many agencies to be a source of complete protein. A complete protein is one that contains significant amount of all the essential amino acids that must be provided to the human body because of body's inability to synthesis them. Phytochemicals, nonnutritive substances that help to maintain good health, are considered functional in foods[12]. Phytochemicals are found naturally in many plant foods, including soybeans. The phytochemicals in soy are primarily isoflavone, although other phytochemicals such as saponins and phytates are also found. The phytochemicals found in soy have been shown to have beneficial effects on the prevention of cancer, osteoporosis, high cholesterol, and other health problems. Soy may be eaten alone, or they may also be incorporated into other food products. The functional components of soy, phytochemicals have also been isolated to add to other food products. Baked goods have been supplemented soy as well as nutritional drinks, nutritional bars, meat substitutes, and other foods. Incorporation of soy into food products may offer increased health benefits but there are also potential problems that may arise. The effect of processing soy may deplete some of their functional components, making them less effective or not effective at all. Soy contains no gluten so when incorporated into baked goods such as bread additional gluten or yeast may be needed to allow the bread to rise.

Soybeans have been consumed for thousands of years, and are important in many different cultures. Soybeans are considered functional foods since they contain high amounts of protein, complex carbohydrates, soluble fiber, micronutrients, and phytochemicals. Soybeans are also the richest source of the phytochemicals called isoflavone[29]. Consumption of soy products such as soymilk and meat alternatives does appear to be on the rise as Americans become more health conscious since soy contains many beneficial nutrients and has been shown to lower cholesterol, prevent cancer and osteoporosis, and may even have a positive effect on menopause and diabetes. Soya bean has 3% lecithin which is helpful for brain development. It is also rich in calcium, phosphorus and vitamins A, B, C and D it has been referred to as "the protein hope of the future". Flavones contained in soya bean are effective cancer preventive agent[16]. Compositing with soya is expected to substantially improve the protein efficiency ratio (PER), in-vitro protein digestibility (IVPD), lysine score and isoflavone content in soya composite formulations[48,40]. With increased awareness a healthy lifestyle based on consumption of functional foods, breads containing whole grain, multi grain or other functional ingredient especially from legumes will increasingly become more important in bakery industry and emerging market.

Aims and objective

1. To formulate and develop functional breads from whole wheat flours composited with soya flour.
2. To optimize the physico-chemical properties of whole wheat bread with different levels of soya flour.
3. To assess the acceptable levels of soybean flour in bread formulations.
4. To fortify bread with soya protein to combat PEM in children.

MATERIALS AND METHODS

The material and method used for the study are as follows:

MATERIALS

Whole wheat flour: - Whole wheat flour was procured from market under the brand name "Ashirwad".

Soybean flour: - SBF was procured from the local market.

Baker's yeast: - Baker's yeast was procured from the local market.

Sugar: - Normal sugar was purchased from the market and used for the preparation of bread.

Salt: - Tata Iodised salt was purchased from the market

Shortening: - Oil was procured from the market under the brand name "Fortune".

METHODS

RAW MATERIAL ANALYSIS

a) Moisture analysis (air oven method): -

The moisture content of wheat flour is determined by AOAC approved method[5].

$$\text{Moisture content (\%)} = \frac{\text{weight of fresh sample- weight of dry sample}}{\text{Weight of fresh sample}} * 100$$

b) SDS sedimentation volume: - Refined wheat flour (5g) and 50ml of water is taken in 100ml stoppered volumetric cylinder. For hydration it was shaken three times for 15 sec at interval of 2 min. 20ml of SDS lactic acid stock solution was added to the cylinder (1 part of lactic acid in 8 parts of water with 50ml of SDS dissolved in water). The sample is inverted four times for 15 sec at interval of 2 minutes. Then the sample was allowed to rest for 40 minutes and sediment reading of sample was noted. (AOAC approved)[5]

c) Gluten estimation: - Wet weight gluten is determined by hand washing method (AOAC)[5]

$$\text{Wet gluten} = \frac{\text{weight of wet gluten}}{\text{Weight of sample}} * 100$$

d) Protein content: - In kjeldahl method of nitrogen estimation, sample is digested in concentrated sulphuric acid. The amount of nitrogen that is determined by this method is multiplied by factor 6.75 for determination of protein content.

Protein content = amount of nitrogen* 6.75

f) Fat content: - Whole wheat flour (2g) was taken in thimbles. Round bottom flask was filled with ether up to $3/4^{th}$ of its volume. It was refluxed for 5 hours at the rate of 5-6 drops /sec. after 5 hours take out thimble and dry in oven for 20-30 minutes at 80^0 C. cool in dessicator and thimble is weighed along with dried sample (AOAC approved)[5].

$$\text{Fat content} = \frac{\text{fat content (g)}}{\text{Sample wt (g)}} * 100$$

g) Ash estimation: - Ignite 3-5g of whole wheat flour in a silica dish at 600^0 C to constant weight. In this method, sample are weighed and charred before ashing on direct flame. Then crucible having charred is placed in muffle furnace at 565^0 C temperatures. Ashing is done for 5-6 hours. Then these crucibles are taken out and placed in dessicator and these are weighed again. The white residue left behind is ash (AOAC approved)[5]

$$\text{Ash \%} = \frac{\text{weight of ash}}{\text{Weight of sample}} * 100$$

h) Dough raising capacity: - Water (45ml) was exactly heated to 40^0 C and it was dispersed in 2.5g of yeast. 35g of flour was taken in a beaker. 1g of sugar was added and mixed in yeast suspension. Mass was made into smooth batter and added to 250 ml graduated cylinder and base level was noted. The rise in level of dough was 15minute intervals for 1 hour. Plot the graph between time and rise in dough volume.

FINAL PRODUCT TESTING

For the final product testing parameters to be tested are moisture, protein, fat, ash and loaf volume. The loaf volume was measured by rape seed displacement method. The other parameters that are moisture, protein, ash and fat are tested by methods approved by AOAC[5] and followed under the topic raw material testing given above.

TEST BAKING PROCEDURE

For the past 20 year, remix baking test has been employed as principle method in the evaluation of baking quality of wheat by the grain research laboratory USA.

The baking method requires high speed dough mixing at both the initial mixing stage and the remix stage which follows a medium long fermentation period. Dough is made from flour water compressed yeast salt and sugar under specified condition of dough temperature, mixing speed and mixing time. Dough is fermented for 2 hours remixed sheeted molded and assessed for absorption and handling properties. It is placed in baking pan, proofed for 40 minutes and baked for 15 minutes. Loaf volume is measured after 25 minutes of cooling and loaves are evaluated the following day for appearance crust color crumb color flavor grain softness and overall acceptance.

Table 1: Standardized recipe of bread making

INGREDIENT	PERCENTAGE (%)
WHOLE WHEAT FLOUR	100
YEAST	6.0
SUGAR	6.0
SALT	1.5
SHORTENING	3.0
WATER	63.5

a) Mixing: Pour all ingredients in a beaker except yeast. Four minutes before schedule mixing time yeast suspension that is 6g of yeast in little amount of water was added for standard recipe. Add this directly to flour in mixing bowl. Start the mixture continuously and mix for atleast 2 minutes. During mixing period, measure ingredient for next test sample. Remove the dough immediately after mixing, round it lightly with hands and place it in a greased fermentation bowl. Place the bowl to fermentation cabinet at 500 C for 30 minutes at 90% relative humidity. Continue other mixing operation as above maintaining time schedule for remaining samples. Place wet cloth over bowls containing dough.

b) Remixing: Remove the dough from fermentation bowl and remix it for 15 seconds and again place it in fermentation bowl for 25 minutes.

c) Intermediate proof: Allow a recovery period for 25 minutes.

d) Remixing: Remix the dough again for 15 seconds.

e) Fermentation: Keep the dough in fermentation cabinet for 15 minutes

f) Sheeting and molding: Remove the dough from fermentation bowl without tearing it and wipe it back and forth on a lightly flour dusted bench top. Spread dough on a clean table by a roller and then and then start molding the spread dough. Turn the end inside and seam the seal. Remove the dough and place it in a baking pan containing numbered slip of paper identifying the sample. The seam of the dough is placed and ends are pushed downward with tips of finger producing pillow-shaped appearance. Place baking pan in proper sequence.

g) Proofing: Proof for 40 minutes at 30^0 C at 90% relative humidity.

h) Baking: transfer the proofed dough from fermentation cabinet to oven (oven temperature maintained at 250^0 C. Remove baked dough from oven after 15 minutes.

Similarly different breads were prepared by incorporation of soya flour at different concentration the method for preparation of bread making is given in table.

MIXING

(Leave for 50minutes at 30^0 C at 90% RH)

↓

REMIXING

(FOR 25 MINUTES)

↓

INTERMEDIATE PROOFING

(FOR 25 MINUTES)

↓

REMIXING

↓

FERMENTATION

(FOR 15 MINUTES)

↓

SHEETING AND MOLDING

↓

FINAL PROOFING

(FOR 40 MINUTES at 30^0C & 90%RH)

↓

BAKING

(FOR 15 MINUTES at 250^0C)

Flow chart for bread preparation

Sensory evaluation: Sensory evaluation is one of the methods used in identifying market acceptability especially in bakery based products. This type of evaluation data are normally analysed statistically, but it is not possible to find out from such analysis the strength and weakness of specific sensory attributes which is responsible for acceptance and rejection of the product, in the statistical analysis of sensory evaluation data, average score of attributes are generally calculated and compared with certain significant level among sample.

Whole wheat bread substituted with soya flour blends were prepared as described in procedure and kept for storage before subjecting them to organoleptic evaluation. A panel of semi trained and untrained panelist from department tested and rated the product according to the hedonic scale as shown in table 2.

Table 2: Hedonic scales

S.No	PREFERENCE	RATING
1	LIKED EXTREMLY	9
2	LIKED VERY MUCH	8
3	LIKED MODERATELY	7
4	LIKED SLIGHTLY	6
5	NEIGHTER LIKED NOR DISLIKED	5
6	DISLIKED SLIGHTLY	4
7	DISLIKED MODERATELY	3
8	DISLIKED MODERATELY	2
9	DISLIKED EXTREMLY	1

RESULT AND DISCUSSION

The whole wheat flour and soya bean that was procured from the local market and was analysed for different parameters and the result for the same is summed up in the table below. The results indicated that the whole wheat flour used for the preparation of functional bread is suitable from the values obtained by parameters such as SDS sedimentation, protein, moisture, ash, fat and gluten content given in the table 3 below. The results show that the soya flour used for bread preparation was very rich in protein than the whole wheat flour used.

Table 3: chemical analysis of whole wheat flour and soya flour

PARAMETER	WHOLEWHEAT FLOUR(%)	SOYA FLOUR(%)
MOISTURE	**7.8**	**5.6**
SDS	**28**	**-**
GLUTEN	**10.67**	**-**
PROTEIN	**12.6**	**38.3**
FAT	**3.65**	**10.2**
ASH	**1.75**	**2.85**

The results don't vary much from the data that has been obtained from literatures[37,48]. The chemical composition of the composite flours has been shown to affect both physicochemical properties and nutritional quality of their products.

Bread characteristics: It is noticed that the addition of soya flour to the whole wheat flour adversely affects the characteristics of bread. It affects the physiochemical properties and dough volume of bread. The increase in concentration of soya flour in inversely proportional to the dough volume as soya concentration increases the dough volume decreases. The dough handling also varies with the increase in concentration of soya flour. The result in the difference in dough handling is summed up in the table 4 given below.

Table 4: Effect of different conc. Of soya on dough handling

Bread sample	C	SB1	SB2	SB3	SB4
Level of soya flour	0%	5%	10%	15%	20%
Dough handling	Smooth	Smooth	Slightly sticky	sticky	sticky

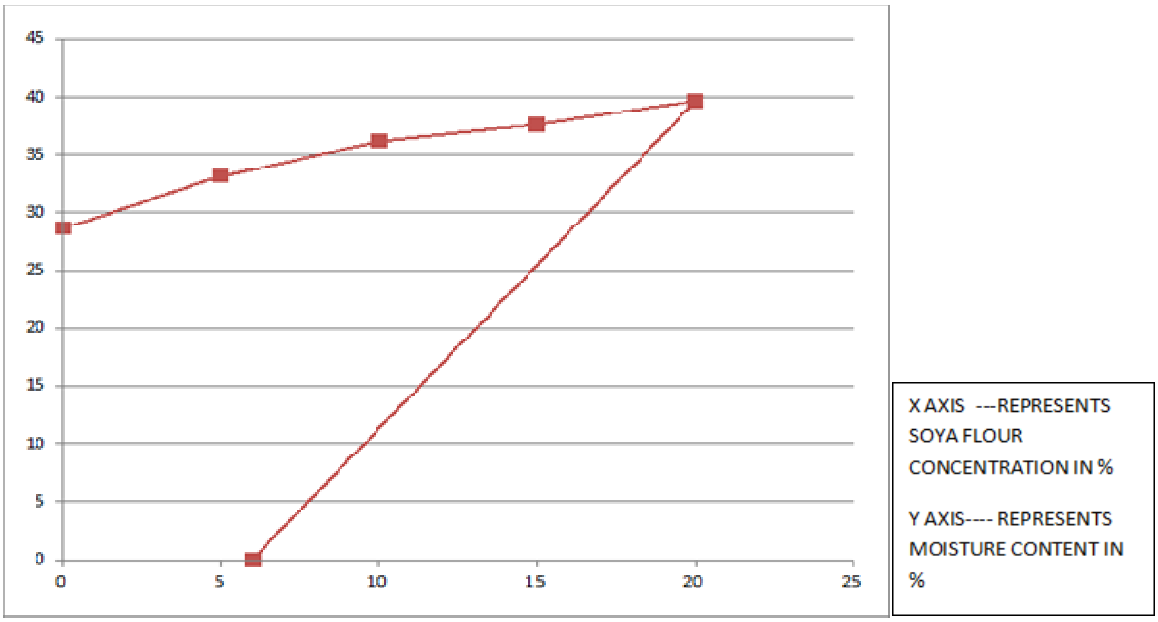
The increased concentration of soya blends affects the physiochemical properties like ash, fat, moisture proteins etc. the proximate values of these parameters were lowest in the control bread and increases as the concentration of soya blends increases. The analysed data for increased parameters are given in the table 5 given below.

Table 5: Proximate result of chemical composition of composite bread

PARAMETERS	CONTROL	SB1	SB2	SB3	SB4
MOISTURE	28.63	33.15	36.16	37.60	39.65
PROTEIN	8.36	9.59	11.03	12.03	12.70
FAT	4.03	4.52	4.79	5.59	6.45
ASH	2.10	2.30	2.54	2.75	2.85

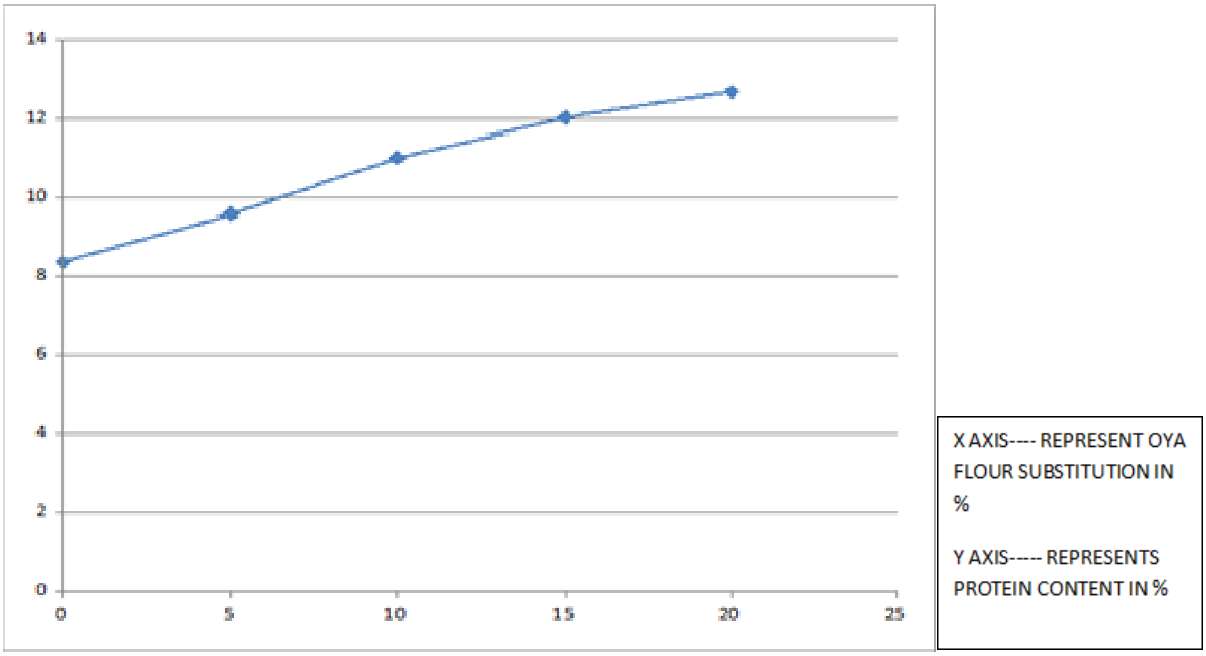
The above results were similar to that obtained from the trends by Serem *et al.*[40] and Islam *et al.*[25]. The above results show the statistical data that the moisture content increases from 28.63 to 39.65% and as the moisture content increases the shelf life of the product decreases and this lead to proliferation of micro-organisms (graph 1).

Graph 1: The study of proximate analysis of moisture content of soya fortified bread



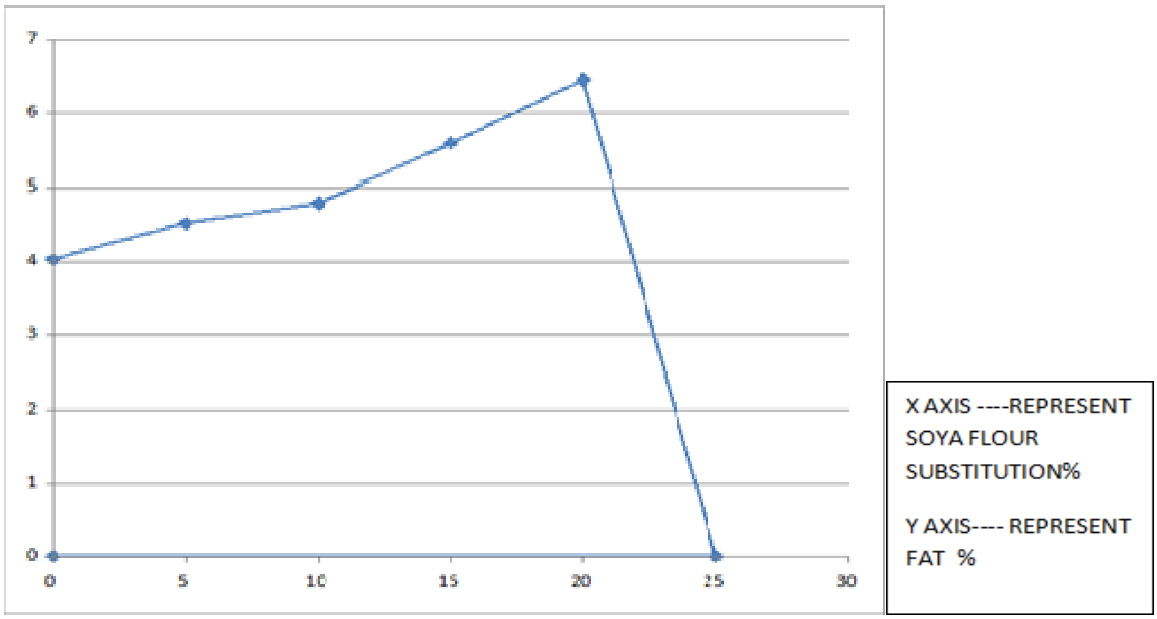
The addition of soya flour in the preparation of bread increases the protein content of the bread. As the concentration of protein rich soya flour increased the protein content of soya flour also increased the protein content raises from 8.36% to 12.70% and it is mainly due to the fact the soya is a rich source of protein and helps to retrieve back the nutritional protein of bread lost during process of manufacturing process (graph 2).

Graph 2: The result of propximate analysis of protein content of soya fortified bread



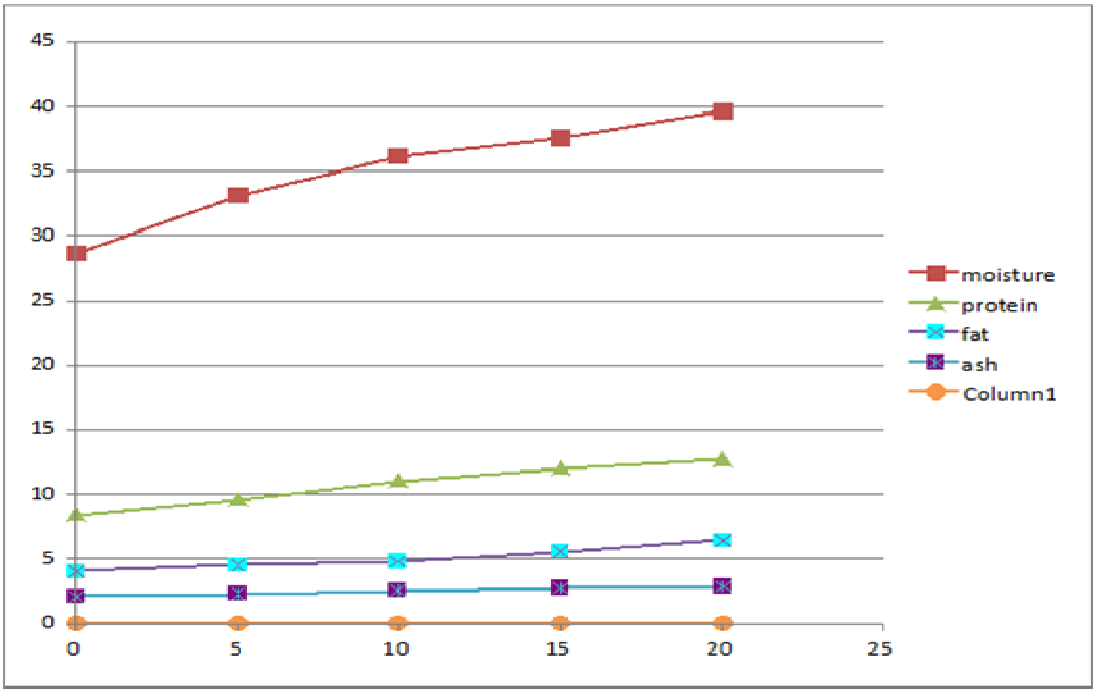
The fat content also rises by increasing the concentration of soya flour. The rise in fat content is from 4.03% to 6.45%. The data has been statistically explained in the graph 3.

Graph 3: The result of proximate analysis of fat content of bread supplemented with soya



The fat content of bread increases as the soya bean flour increases. Soya bean from which soya flour was produced is an oil seed and is the main contributor to the increase in oil content that is fat of the product. The complete analysed data of the values obtained from the data collected above is summed up in the following graph 4:

Graph 4: Analysis of all physio chemical properties affected by addition of soya flour at different concentration to whole wheat bread



The addition of soya flour to whole wheat flour depresses the loaf volume of the bread. However the percentage decrease in loaf volume was much greater in case of soya bean fortified with 20% soya blends and this can be identified in figure 1.

Fig. 1: Volume of bread

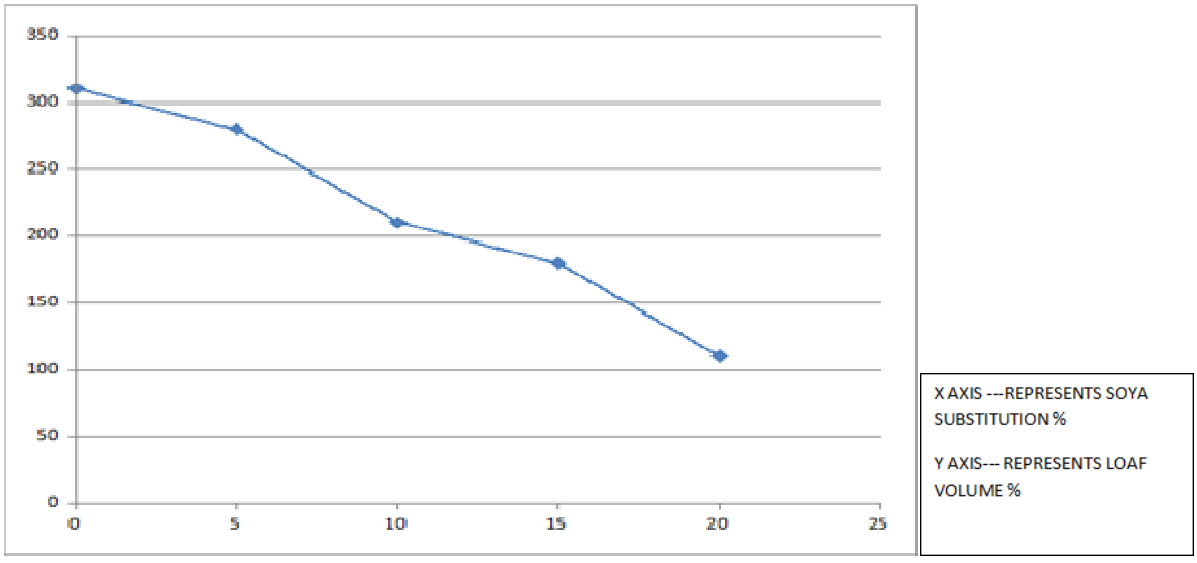
Control	SB1	SB2	SB3	SB4

Bread volume decreased as level of substitution of soya flour increased. The effect of soya bean flour substitution on loaf volume of whole wheat bread was measured by rapeseed displacement method and graphically represented in the graph 5 given below.

Table 6: The result of bread characteristics

PARAMETERS	CONTROL	SB1	SB2	SB3	SB4
LEVELS	0%	50%	10%	15%	20%
BREAD VOLUME	310	280	210	180	110

Graph 5: Effect of soya flour on loaf volume of bread



Sensory evaluation

The bread formulated from addition of different concentration of soya blends in whole wheat flour was evaluated for sensory properties by a panel of semi trained panelist. The sensory properties that were analysed for appearance, flavor, texture, crumb and crust of the final bread were analyzed.

The results for the sensory evaluation containing different soya flours are compared with the control bread summed up in the table 7.

Table 7: Sensory mean score card of bread samples

PARAMETERS	CONTROL	SB1	SB2	SB3	SB4
COLOUR AND APPEARANCE	7.9	7.9	8.0	7.7	7.4
FLAVOUR	8.2	8.1	8.3	7.2	6.8
TEXTURE	8.1	8.2	8.2	7.6	7.1
CRUST	7.5	7.6	7.6	7.4	6.9
CRUMB	7.9	7.9	7.7	6.5	5.4
OVERALL ACCEPTABILITY	7.82	8.0	7.9	7.0	6.5

The result of the bread color and appearance shows the difference when compared with the control bread. The score for texture of composite bread samples decreased as the soya content increases as the addition of soya flour to the wheat flour affects the rheological properties of dough and hence affect the quality of the final bread. Hence when SB 4 compared with control had poor texture. During baking condition, the state of bread components as starch, protein, gluten, amount of water absorbed during dough mixing all contribute to the final texture of the formulated bread.

The addition of soya flour has adverse effect on formation of whole wheat bread and had great variation in the flavor of the product. As the concentration of soya flour increases its beany aroma and flavor in bread get more pronounced. In soya beans enzymatic break down by lipoxygenase or auto oxidation of linoleic acid linoleic acid produces hydroperoxides such as ketones, aldehydes and alcohol that may be responsible for the beany flavor which discourages soy consumption. Upto 10% the bread does not have any beany flavor but as concentration increase its beany flavor also become more pronounced and becomes unacceptable by the consumers.

The crust and crumb of the bread were also affected by the soya blends the crust of the bread becomes darker as the concentration of soya flour increases. The crumb also dealt with the increasing soya flour concentration. The data given above shows that crumb becomes sticky on addition of soya flour.

When the sensory evaluated scores were compared with the control and with one another the results show that the SB2 bread that is with the 10% concentration of soya as acceptable by the consumers. The SB2 bread has good color different from the control and better from SB3 and SB4 concentrated breads. The flavor of SB2 was also liked by the consumers as the control was similar to the normal bread available in the market and SB2 has unique in flavor. And the crumb was also not as stickier as SB3 and SB4.

CONCLUSION

From the present study it is concluded that bread fortification with different concentration of soybean flour can be done up to 10% without affecting consumer acceptable limits of bread. Composite bread with soybean flour substitution were found to be nutritionally rich as the addition of soya flour to the whole wheat flour for bread preparation increase the protein and fat content of the bread. These breads are nutritionally superior as the level of lysine content increased due to increased protein content. As the moisture content increases with increase in soya concentration the risk to microbial spoilage hence shelf stability also increases. Therefore whole wheat bread was considered more suitable than soya substituted breads.

The composite bread serves as functional food because of high protein content of soya. Soya blended bakery product with the general advantage of ready availability and consumability may serve the purpose of better nourishment without changing the food habits of the consumer. The further research should be conducted on improving the overall acceptability of soya substituted breads. Hence there is also a need to improve mixing ingredient to improve the quality of functional bread.

Further research should be conducted with addition of extra fiber to the soya bread. The fiber addition usually depresses the loaf volume making its appearance unacceptable.

Another research with addition of isoflavones should also be conducted. Isoflavones are beneficial in reducing the risk of cancers specially breast cancer.

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