The Effect of Microwave Treatment on Conventional *Feed Ingredients*

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THESIS

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in

Plant Biotechnology

Submitted by

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Date:
Place: Varanasi ***(Alla Yaswanth Naveen Kumar)***

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ABBREVIATIONS

SB	Soyabean	ANF	Anti Nutritional Factor
PDI	Protein Dispersibility Index	SPC	Soybean protein Concentrate
SBM	Saybean Meal	SPI	soy protein Isolate
NPN	Non-Protein Nitrogen	Aas	Amino Acids
DM	Dry matter	CP	Crude Protein
CF	Crude Fiber	AIA	Acid Insoluble Ash
ADF	Acid Detergent Fiber	NDF	Netural Detergent Fiber
mg	Milligram	g	Gram
β	Beta	±	plus or minus
µ	Micro	%	Percentage
DORB	De-Oiled Rice Bran	RB	Rice Bran
ºC	Degree Centigrade	ANOVA	Analysis of Variance
cm	Centimeter	W	Watt
APS	Ammonium Per Sulfate	SDS	Sodium dodecyl sulfate
PAGE	polyacrylamide Gel electrophoresis	L	Liter
mM	milli Molar	AAE	Ascorbic Acid Equivalent
GAE	Gallic Acid Equivalent	QE	Quercetin Equivalent
KDa	Kilodaltan	TPC	Total phenolic content
TFC	Total flavonoid Content	DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
TAOA	Total anti-oxidant activity	DW	Distilled water

Chapter1

INTRODUCTION

Soybean (SB) usually referred as *Glycine max* belongs to the family Fabaceae. Soybean originated in East-Asia and probably it was first domesticated nearly 5000 years ago (Carter et al., 2004). A few plant species, introduced from Asia to North-America during the first decades of the last century, carried the genetics of North-American cultivars, which has undergone almost eight decades of extensive breeding to develop the elite cultivars, now cultivated widely in North America and elsewhere (Hyten et al., 2006; Specht et al., 2014). It is an annually cultivated self-pollinated diploid legume that holds great nutritional and economical utility among the beans grown world-wide. It is an erect and productive crop that acts as a key source of vegetable protein for millions of people along with harbouring a wide array of chemical ingredients to serve the nutritional perspective. It is a protein-rich, high oil containing resource that is frequently employed as human food to animal feed ingredient as well as base material for diverse industrial products. A pivotal step to utilize soybeans is to separate oil from protein and fiber. This has been achieved successfully by using either solvent extraction or expeller pressing—both result in oil and protein-rich meals. European Union, South and Southeast-Asia, and Mexico are the major countries soybean importing soybean, accounting for almost 80% of the soybean global export value, that includes seed, oil, meal, and biodiesel (FAOSTAT, 2013–17). After wheat, maize, and rice Soybean is the fourth most important grain crop that cultivates worldwide. According to market standards has moisture content of 13%, a soybean seed can be processed into about 19% oil fraction (used for cooking, biodiesel, and other industrial uses), leaving behind almost 68% meal fraction (used as animal feed). The meal can be further processed into human consumables such as milk, tofu, and flour.

Whole raw SB is used as a source of protein and energy for lactating cows; however, SB contains different anti-nutritional factors (ANF) that limit the nutritive

value. For instance, urease, which hydrolyzes urea to ammonia, and trypsin inhibitors, which reduce protein digestion in the small intestine. The grounded or cracked soybean is usually not recommended in diets due to the presence of urea and enzymes such as lipase, lipoxidase, and peroxidase which alter the quality of fatty acids. These enzymes can be inactivated to enhance the nutritive quality by proper heat treatment without affecting the other parameters of digestibility. Grinded or rolled soybeans in diet reduce the amount that passed through the intestine and increases the DM digestibility and ruminal protein digestibility. Ruminal fiber digestibility declines when ground or rolled SB is fed due to the polyunsaturated fatty acids that are toxic to the ruminal microbes and the fat may physically coat the dietary fiber preventing the microbes from attaching to fiber. Cracking SB to half and quarter sizes does not alter ruminal fiber digestion and results in higher milk yield than that achieved with SB rolled to quarter or smaller sizes or ground. Cracked or grounded SB should be fed to the animal quickly otherwise, the probability of fatty acid oxidation increases. Roasting reduces the ruminal protein degradability and inactivates the enzymes that interfere with digestion. The effectiveness of roasting heat treatment can be measured using the protein dispersibility index (PDI). The PDI denotes the solubility of a protein in water that is usually used in the soybean product industry. Low PDI indicates better digestible protein content in the treated feed. The PDI of underheated SB is greater than 14%, for marginally roasted is 11-13% and for optimally roasted SB is 9-11%. The estimated ruminal undegradable protein of optimally roasted SB is more compared to raw SB i.e. for roasted SB is 60% and for raw SB is 27% (Bernard, 2011).

Soybean Meal (SBM) also known as Dedoiled Soybean cake is one of the major by-product of soybean processing. Soybean Meal is an irregularly shaped flake, pale-yellow or light brown in colour and smells like roasted soybean. The general nutrient profile of SBM is depicted in Table 1. Soybean meal is a major protein source in feed ingredients. It is extensively used as a feed ingredient for livestock and poultry. In terms of consumption, the soybean meal takes up over 60 percent of the world's total consumption of protein meal. Soybean Meal is high in protein and has a good amino acid profile, making it ideal for all livestock (Table 2).

The rapid increase in animal-based products stimulates the demand for quality protein feed. There are various types of animal feed available in the market such as rapeseed and cottonseed meal, however both ingredients cannot be used in feeds unless they are detoxified. Therefore the most widely used protein-rich quality meal in India is soybean meal. The major reason for the popularity of SBM is the unique composition of amino acids (AAs) which complements the AA compositions of many cereal grains. While SBM is the most popular soybean product utilized in livestock diets, other products are also being used to a varying degree. These products include full-fat soybeans, Soy Protein Concentrate (SPC), Soy Protein Isolate (SPI) soy-bean oil, and soybean hulls. The crushing of plant and removal of hulls increases the protein content. So, dehulled SBM contains more protein content as compared to non-dehulled SBM. To differentiate between dehulled and non-dehulled SBM measuring of fiber content is necessary. Dehulled SBM contains 3% crude fiber whereas non-dehulled SBM contains more than 6% crude fiber. The less fiber and ash content indicates higher content of protein, higher energy, lysine, methionine and other amino acids. Sometimes higher protein content also results due to addition of non-protein nitrogenous compounds such as urea, ammonium compounds, or even melamine. It is necessary to measure the non-protein nitrogen (NPN) to know whether the feed is adulterated or not. SBM also contains of ANF such as trypsin inhibitor and lectin. To inactivate such ANFs SBM can be subjected to heat treatment. Under-treated SBM still exhibits some ANF whereas over-treated SBM damages the amino acids. So, optimum heat treatment is necessary to inactivate the anti-nutritional factors.

Table1: percentage of nutrient content in SBM

S.No	Nutrient	% in SBM
1.	Dry matter (DM)	79-88
2.	Crude protein (CP)	43.8-49.9
3.	Ash	5.6-7.2
4.	Crude fat	0.55-3.0
5.	Crude fiber (CF)	4.3-7.2
6.	ADF	12.3-18.9
7.	NDF	8.9-11.9
8.	N-free-extractive	34.3
9.	Starch	5.51

Table 2: % of essential AA content in SBM

S.No	Amino Acid (AA)	% of AA in SBM
1.	Arginine	3.49-3.78
2.	Cystin	0.66-0.75
3.	Histidine	1.21-1.32
4.	Isoleucine	2.15-2.78
5.	Leucine	3.66-3.92
6.	Lysine	2.99-3.22
7.	Methionine	0.6-0.69
8.	Phenylalanine	2.35-3.0
9.	Threonine	1.89-2.03
10.	Tryptophan	0.66-0.75
11.	Valine	2.24-2.67

Soybean meal (SBM) is commonly used as a protein supplement in dairy rations. SBM is palatable and contains large quantity of amino acids (AA). It used as standard to determine the value of other supplements. SBM is more limiting in essential amino acid methionine than lysine. SBM is heated or chemically treated to

increase the flow of dietary amino acids to the small intestine and reduces ruminal protein degradability. The commercial procedures which are conventionally used to produce heat-treated SBM includes non-enzymatic browning of dehulled, solvent-extracted SBM, cooker-expeller processing of SB, extruder-expeller processing of SB, and cooker processing of dehulled, solvent-extracted SBM. The flow of amino acids to the small intestine can be increased by heat treatment of SBM which results in higher milk yield. The flow of dietary amino acids to the small intestine can also be increased by treating SBM with chemical such as sodium hydroxide or formaldehyde, sulphate liquor and xylase but this flow doesn't include essential amino acids due to reduced microbial protein flow. Positive responses in milk yield have noticed with treated SBM when Lucerne is used as primary silage compared to maize silage. Compared to solvent-treated SBM, heat-treated SBM shows higher milk yield (Bernard, 2011).

Rice is commonly referred as *Oryza sativa* belongs to the grasses family poaceae. Rice is annual monocot cereal crop majorly grows in tropical areas. As cereal grain it widely consumed as staple food by most of the world population especially in Asia and Africa. It mainly grows in areas of high rainfall and low labour cost because rice cultivation needs intensive labour and high irrigation. Even in steep hill and mountain region rice can be cultivated by water-controlling terrace system. The parent species of rice native to Asia and Africa, its trade and exportation made common crop in many places world-wide. As per 2010 statistics rice production and consumption is responsible for about 4% emission of green house gases. Rice serves as major energy food for 17 countries in Asia and Pacific, 9 countries in North and South America and 8 countries in Africa. It provides 20% of worlds dietary energy supply which followed by wheat 19% and maize 5%. A detailed analysis shows that nutritive value varies depending upon various factors such as strains of rice i.e. white, brown, red and black, soil where it grown, processing of rice and its preparation before consumption.

Table3: Nutrient content of rice per 100g dry weight

S.No	Nutrient	Nutritive value (in gms)
1	Energy	1736
2	Protein	8.1
3	Fat	0.8
4	Carbohydrate	91
5	Fiber	1.5
6	Sugar	0.1

The rice also contains various minerals such as calcium, iron, manganese, magnesium etc. The mineral value is explained in table 4 below

Table4: Mineral content of rice per 100g dry weight

S.No	Minerals	Mineral value (in mg)
1	Calcium	32
2	Iron	0.91
3	Magnesium	28
4	Phosphorous	131
5	Potassium	131
6	Sodium	6
7	Zinc	1.24
8	Copper	0.25
9	Manganese	1.24
10	Selenium	17.2

The rice has various by-products such as broken rice, husk, bran layer, rice bran and de-oiled rice bran (DORB). These by-products are prepared by different processing methods. Rice bran is produced by processing of paddy through whiteners and milling. DORB is prepared by extraction of crude oil from rice bran. The chemical composition of Rice bran and DORB mentioned in table 5 (Islam, 2018).

Table 5: Chemical composition (g/100gDM) of RB and DORB

S.No	Parameter	Rice bran (RB)	DORB
1	Dry mater	90.00	89
2	Crude protein	14.61	17.85
3	Crude fiber	11.05	14.60
4	Ether extract	17.20	1.60
5	Ash	9.9	14.3
6	NFE	47.24	51.45

De-oiled rice bran is important feed for cattle, poultry and pigs. DORB is the cheapest feed that available throughout the year. It supply in the diets as major supplement along with oilcakes in livestock ration. As it is fat free or polished rice bran it is good source of protein and fiber. It considered as chief feed for complete development of livestock. However in recent times the consumption of DORB increased which increased its cost also. Different alternative feeds are using to replace the DORB due to increase in demand and cost. So it is necessary to increase production and use of available DORB effectively. To increase the digestibility of DORB, it is subjected to various treatments such as boiling, fermentation,

autoclaving and microwave treatment. The microwave treatment of optimum frequency and time period break down the covalent bonds and increases the protein and fiber digestibility without affecting the biochemical and anti-oxidant properties.

Maize also known as *Zea mays* belongs to family poaceae. It is cereal grain first domesticated about 10,000 years ago by indigenous people of Mexico. Maize is cultivated throughout the world and compared to other cereal grains it has higher grain weight. There are majorly six types of maize varieties such as dent corn, flint corn, pod corn, pop corn, flour corn and sweet corn. Generally sweet corn kernels consumed by a human which has high sugar content. Other field corn varieties used as feed for animals. Maize also used in production of ethanol and other bio-fuels. Maize is cold tolerant plant and cultivated in temperate regions during spring season and due to its deep swallow root system for water it depends upon soil moisture. As it consists of C4 fixation mechanism more effective in water use compared to C3 plants such as soybean and alfalfa. Maize provides support for bean crops in-return legumes provide nitrogen by nitrogen-fixing bacteria (*Rhizobia*) to maize. Many maize varieties grown in United States and Canada are hybrid. To develop resistance to pests and glyphosate genetically modified varieties are developed. The maize is harvested as grains matured and moisture content is decreased. Later on harvesting of the cobs remaining plant is used as silage for livestock. The grains are dried and stored in bins. Care should be taken that moisture content of the grains is not too high which leads spoiling. The main reason for using maize as staple food is high nutritive value with high levels of carbohydrates, oils and other valuable proteins. Maize is good source of dietary fiber and protein while low in fat and salt content. Maize is naturally deficient in two essential amino acids such as lysine and tryptophan. Maize has tremendous composition of carotenoids. The carotenoid beta carotene which is a precursor of vitamin-A naturally converts in plant body to vitamin-A which is essential for human health especially for vision and anti-oxidants. The table given below shows the nutrient content of maize.

Table 6: Nutrient content of maize per 100 g dry weight

S.No	Nutrient	Nutritive value
1	Dry matter	90
2	Protein	10.4
3	Fat	5.3
4	Starch	82
5	Fiber	8.1
6	Sugar	0.7

The maize was subjected to various thermal treatments such as hot temperature, pasteurization, water bath canning, pressure canning, drying and microwave sterilization. Maize flour is subjected to microwave treatment to improve starch and protein digestibility.

Microwave sterilization is thermal processes. The microwave oven passes ionizing radiations of 2.125 GHz. The frequency of microwaves lies between radio waves and infrared waves. Microwave heating takes place due to polarization of electromagnetic radiation frequencies between 300 MHz and 300 GHz. It provides energy to the processed material under controlled temperature and pressure which inactivates and destroys harmful microbes and makes material sterilized. Prolonged exposure to microwave frequency should affect the food quality. The microwaves interacted with water molecules and charged ions which reduce the moisture content of the material. Microwave treatment improves storage period of material. Microwave treatment significantly reduces the thermal treatment time compared to the other conventional treatments. The microwave sterilization improves the colour,

texture and other attributes of the food. US Federal Communication Commission (FCC) allocates 915 MHz and 21250 MHz bands for industrial and domestic microwave heating applications. The microwaves penetrate about 1/3 to 1 inche in to the treated material from all the sides. So the microwave oven is of about the limited size only. So it is best to cook small quantity of food in microwave oven and increases in quantity of food may decreases efficiency of treatment.

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Chapter 2

REVIEW OF LITERATURE

The success of microwave treatment to the conventional feed ingredients depends upon wide factors such as treatment time, wattage, feed type, moisture content in feeds, etc. The objective of microwave treatment was to increase the digestibility of the feed ingredients with minimal destruction of the nutritive value.

Szabó *et al.* (1998) worked on optimization of microwave treatment in soybean to reduce the enzyme activity such as urease along with reduction in ANF trypsin inhibitor. They mainly investigated the effect of microwave treatment on whole soybean. The entire experiment was carried out using Labotron 500 vacuumable microwave equipment without focusing on physical parameters. The aim of this research was to develop experimental design to decrease anti-nutritive constituents by microwave treatment and to find out optimum conditions for treatment.

Li *et al.* (2002) worked on developing the ultrasound and microwave-assisted extraction of soybean oil. The main objectives of the research were to determine the effect of microwave on soybean oil extraction, effect of ultrasound on soybean oil extraction and study the effect of different solvents on soybean oil extraction. Two soybean varieties (TN 96-58, N 98-4573) were used in the experiment. Oil was extracted using different solvents [hexane, isopropanol, and mixed solvent (hexane: isopropanol 60%:40% v/v)] by either using a traditional procedure without ultrasound application or sonication by an ultrasonic probe at intensity levels ranging from 16.4 Wcm-2 to 47.6 Wcm-2. It was found that higher sonication intensity allowed for more efficient oil extraction (faster and greater oil yield). When using hexane:isopropanol solvent mixture the oil yield extracted was higher. Microwave assisted extraction has enhanced both oil yield and extraction rate. The oil extraction yielded 0.45 g more in microwave-assisted extraction compared to the control soybean. Extraction rate was also enhanced with increasing in treatment time.

The effect of microwave roasting on protease inhibitor activity and soluble protein content and composition in cracked soybeans was investigated by **Barać *et al***. (2005). Soybeans of Hodgson var. were cracked to 1/6-1/8 of the whole bean size. Soybeans were dehulled and exposed to microwave frequency of 2.450 MHz. soluble protein content of hexane defatted solution was determined by SDS-PAGE. Residual protease inhibitor activities and isoinhibitor composition were also determined. The duration of microwave roasting had strong influence on soluble protein content and polypeptide composition. Residual protease inhibitor activities and isoinhibitor composition were also determined. The trypsin inhibitor activity reduced to 13.33% of the initial value of two minutes roasting. Kunitz (KTI) and Bowman-Birk (BBI): two inhibitors were responsible for residual inhibitor activity. Characterization of microwave treated samples were dominant in glycinin content; and high stability of acidic (-A1,2,3-, -A5-) and basic (-B1,2,3,4-) glycinin subunits were established.

The effect of microwave treatment on the trypsin inhibitor and triglycerides in soybeans has been elucidated by Yoshida & Kajimoto, (1988). Soybeans of different moisture content (8.6, 24.3, and 49.7%) exposed to microwave frequency of 2,450 MHz. The trypsin inhibitor activity was completely inactivated in soybean of 24.3% moisture content soaked for 1 hr and treated for 4 Min. The trypsin inhibitor activity in soybean of 49.7% moisture content and soaked for 5 hrs were not completely inactivated. Both ultrasound and microwave treatment had a positive effect on soybean oil extraction. Soybean oil yield was increased in mixed solvent extraction using hexane and isopropanol.

Conventional and microwave treatment on soymilk for the inactivation of trypsin inhibitors and subsequent in-vitro protein digestibility were compared by **Vagadia** *et al*. (2018). Soymilk which contains serine protease inhibitors lowers its nutritional value and digestibility. Soymilk processing techniques have been developed for the elimination of trypsin inhibitors and lipoxygenase. Optimum conditions for microwave treatment of soymilk were developed to increase the digestibility as compared to conventional thermal treatments. The microwave

treatment frequency of 2.45 GHz for 2, 5 and 8 min and temperatures of 70 °C, 85 °C and 100 °C were investigated and compared to conventional thermal treatments at the same temperature for 10, 20 and 30 min. To design and optimize experimental conditions response surface methodology has been used. Trypsin inhibitor activity reduced by 1% in microwave treatment and 3% in conventional thermal treatments and digestibility was increased by 7% in microwave treated soymilk and 11% in conventional treatments.

The effect of different thermal and non-thermal treatments on total phenolic and antioxidant content of soybean was studied by **Kumar & Rao**, (2019). Different treatments had been applied to inactivate anti-nutritional factors and simultaneously their effect on total phenolics (TP) and antioxidant capacity (AOC) were evaluated. Autoclave boiling, High pressure processing (HPP), and Microwave treatments were employed to soybean at soaking condition and simultaneously their TP and AOC were estimated. HPP not exhibit any significant effect on the TP and AOC content. Autoclave boiling exerted severe effect and lead to degradation of the bioactive ingredients by more than 50%. The microwave treatment also affected TP and AOC content but not severely. In HPP at high pressure treatment time did not affect TP concentration. The concentration of phenolic content at 400 Mpa for 10 min was observed to be 1.88 mg/100g of the sample and was almost similar at 400 Mpa for 5 min (1.87 mg /100g of the sample) condition. The TP concentration majorly impaired in autoclave boiling treatments and observed to be 1.07 mg/100 g of sample and while AOC was 94.77 mg/100 g of the sample.

The effect of microwave irradiation of whole soybeans in ruminant nutrition and its protein and carbohydrate metabolism in-vitro and in-situ was investigated by **Golshan** *et al.* (2019). In ruminant nutrition whole soybean serve as main source of protein. Diverse processing conditions have been implemented for ruminant protein protection. The effect of microwave irradiation [900 W; 2, 4 and 6 min] on quality, ruminal degradability and in-vitro intestinal digestibility of the available soybean crude protein was determined. The experiment was performed with seven treatments along with control. The treatment time included 2, 4 and 6 min of microwave

irradiation on whole and ground soybeans. Triplicates of the sample were incubated in the rumen of three cannulated Holstein steers for up to 48 hr. Microwave irradiation increased metabolizable protein content, neutral detergent insoluble nitrogen and resulted in a lower effective rumen degradability and in-vitro gas production. Without exhibiting any negative effect on protein and carbohydrate availability, microwave irradiation of ground samples for 4 min increased the metabolizable protein content.

Extraction of soluble protein and sugar from defatted soybean meal and jackfruit peel was carried out using microwave-alkaline hydrolysis with varying concentration of sodium hydroxide (0.04–0.11 M) and different wattage-time (300 W microwave power, 2–11 min) combination by **Roslan** *et al.* (2022). The highest protein (5.31 mg/mL) and sugar concentrations (8.07 mg/mL) with > 75% recovery was achieved at optimum conditions of 0.084 M NaOH concentration (100 mL), for 8.7 min at 300 W microwave power level. Analysis of nutrient utilization dynamic by Enterobacter hormaechei strain 40a was carried out. Both detoxified hydrolysate (using activated carbon) and raw were correspondingly biocompatible with Enterobacter hormaechei strain 40a ($P > 0.05$) resulting in maximal cell counts of > 10 log CFU/mL. The optimized hydrolysate was prepared as an additive in molasses-alginate bead encapsulation of strain 40a and evaluation of potassium solubilization and phosphate performances of the encapsulated strain exhibit significant result compared with free cell counterpart.

The effect of heating of soybean seeds through microwave radiation on the separability of the shell was carried out by **Mkhitaryants**, *et al.* (2018). Soybean variety 'Vilana' harvested in 2017 was the object of the research. The microwave treated soybean seed shell was investigated and treatment time varied from 30 to 50 seconds. In seed samples temperature was determined and the grains were subjected to crushing next day. The resulting crusher was disassembled into 3 parts whole seeds, core and the shell. Then mass fractions of the separated shell and the shell remaining in the crusher were determined. The microwave radiation has a significant effect on the separability of the shell, even for a short period of heating. The non-

treated seeds contained processed core is 3.0% while in treated seeds at 70ºC and 80ºC the shells were 1.6%, and 1.3% respectively. It represents that with an increase in the heating temperature, the efficiency of the shell separation increases. To prevent the denaturation of protein, the heating temperature was limited to 70ºC.

Improvement in the quality of the soybean by-products by physical methods during its use in bakery technology has been done by **Wang** *et al.*(2020). The by-products of soybean had high nutritional value but their use is limited due to rough taste and a low content of soluble dietary fiber. The main objective of the research was to study the secondary raw materials of soybean processing (SMSP) and soluble dietary fiber, trypsin inhibitor, bakery products, pastries and the effects of physical methods of raw materials processing. Analysis of results depicted critical influence of physical factors of SMSP on technological, nutritive value and finished products. There was a significant effect of high pressure on soluble dietary fiber and functional features of legume wastes. In comparison to untreated, the treated SMSP at 400 MPa and 60℃ under high pressure, the content of soluble dietary fiber increases by 8 times. Microwave treatment has a strong penetration power. Microwave treatment is an effective way to inactivate the activity of a protease inhibitor in cracked soybean. Two minutes of treatment reduces trypsin inhibitor activity to 13.3% as compared to the normal.

The Effects of microwaving, moist and dry heating on ruminal degradability of protein and dry matter in soybean meal was studied by **Rezaii & Bayat kohsar** (2019). To evaluate the effect of autoclaving, roasting and microwaving on gas production and *in-situ* rumen degradability of soybean meal, two experiments were conducted. Autoclaving was done at 121^0C for 20 Min, Microwaving for 2, 4 and 6 min and roasting at 140℃ for 30 and 60 min, and at 160℃ for 30 and 60 min. A gas production trail was performed by collecting rumen liquid from fistulated sheep. Gas production cumulatively recorded at 2, 4, 6,8,12, 24, 36, 48, 72 hrs after incubation. After 24 hrs of incubation organic matter digestibility (OMD), net energy (NE), metabolizable energy (ME) and short-chain fatty acid (SCFA) were calculated in gas production trail. For *in situ* experimental technique three Dalaq breed fistulated

sheep of average weight (54 kg) are kept in separated cages and fed to maintained level. Samples of both treated and untreated were grounded and passed through 3 mm mesh and 5 g of each sample were transferred to nylon bags. The results concluded that there was significant effect that gas production increased with increase in heating duration in roasted treatments. In autoclave treatment lowest amount of gas produced. In microwave (6 min) and autoclave treatment organic matter digestibility significantly decreased as compared to heating. The concentration of SCFA significantly decreased in microwave treatment as compared to other treatments. *In situ* results showed that SBM processing significantly decreased protein degradability and rate of dry matter degradation. Finally results concluded that processing treatments (autoclaving, dry heating, and microwaving) of SBM decreased protein degradability and rate of gas production.

The effect of heat-processing on the soluble protein content of soybean flour was studied by **Caprita** *et al.* (2010). The reduction in protein nutritive quality and digestibility were important and frequently observed as the effects of heat treatment. Trypsin inhibitor activity was decreased by heating treatments and overheating may destroy some heat sensitive amino acids which reduce soy nutritive value. The principal objective of this research was to evaluate protein solubility of soybean flour (SBF). Heat processed SBF was forced into air oven at 120^0C, and exposed to microwave at 2450 MHz frequency. Araba and Dale procedure (soybean proteins in 0.036 M KOH) was used to determine protein solubility. A comparative study was performed for two treatment methods, between oven heated at 120^0C and microwave treatment at 800W. Microwave treated SBF for 3 min at 800W and oven heated for 20 min at 120^0C decreases protein solubility below 50%. Heating SBF 30 min decreases solubility to 21% and microwave treatment for 5 min decreased solubility to 18.7%. Protein solubility rapidly decreased in microwave treatment than in conventional thermal heating. Therefore it is evident that short microwave treatment improves the nutritive quality of SBF.

The biochemical evaluation of poultry byproduct meal as a substitute for soybean meal was done by **Kamali** . *et al.* (2019). The objective of this experiment

was to investigate the role of nitrogen in livestock potential and use of byproducts instead of conventional feed source to reduce the cost. In the experiment seven treatments of different feeds of varying protein content was used. Soybean meal of varying protein content i.e. soybean meal (1-100%, 2-67%, 6-33%, 5-67%), poultry slaughter house waste powder (33%, 67%, 4-100%), microwave treated poultry slaughter house waste powder (33%, 67%, 7-100%) were used. Four lambs are fed individually for each treatment, Totally 28 animals were tested individually after 70 days of trail. The various parameters such as fat yield, economic efficiency, apparent digestibility, blood parameters and carcass analysis of treatments were evaluated. The results showed that the replacement of soybean meal with different levels of conventionally treated and microwave-treated slaughterhouse waste had no effect on feed performance, including: dry matter intake, daily gain, feed conversion ratio and dietary intake. Treatments 2 with the lowest digestibility (50. 90%) had a significant difference in comparison to other treatments (P<0. 05). Feed costs per kilogram of weight gain for treatments 3, 4, 6, 7 and 4 were reduced by 15, 7, 6 and 5 percent, respectively, compared to treatment 1, which contained 100 percent protein sources of soybean meal. The results of this study showed that the slaughterhouse waste pests of conventional type (without treatment) and type of microwave processing had no effect on fattening performance (weight gain, conversion ratio and food efficiency) of lambs. Furthermore, its replacement levels with soybean meal, up to 100%, while maintaining the characteristics of fattening traits, have a favorable economic return.

Blood biochemical analysis of broilers fed with differently thermal processed soybean meal has been done by **Nahavandinejad** *et al.* (2014). A 42-day feeding trial was carried out to evaluate the influences of differently thermal processed soybean meal on the broilers blood biochemical parameters. A total of 200 male birds of Ross strain were allocated into five different diets formulated using differently heat-treated soybean meals, with ten birds per treatment and per replicate. Diets contained: raw soybean (controls), autoclaved for a short (121°C, 20 min; Aut1 group) or medium length period (121°C, 30 min; Aut2 group) soybean meal, microwaved soybean meal (46°C, 540 Watt, 7 min; McW group) and browned

soybean meal (120°C, 20 min; Brn group). Blood serum metabolites showed that all treated diets presented lower lipid metabolism makers and higher protein metabolism markers. Broilers showed increased final body weight when fed with heat-treated meals compared to the control. Results suggested that thermal treatments altered the lipid metabolism in broilers that might result a decrease in abdominal fat deposition. Comparison of the results for all the treated groups showed that Aut2 treatment is the most suitable method for soybean thermal treatment processing; in contrast, the Aut1 treatment had the closest results to the control group.

The analysis of commercial and non-commercial peroxidases activity under ultrasound and microwave treatment was evaluated by **Simone** *et al.* (2017). The study was aimed at evaluating the activity of peroxidases after ultrasound and microwave radiation exposure as an alternative to the wastewater treatment improvement. The extraction of peroxidase was held from the agro-industrial by-products, rice bran and soybean meal. The initial conditions for enzyme extraction were based on the method described by Cardinali et al. (2011). To evaluate the effects of ultrasound on the enzymes activity, tests were performed in the presence and absence of ultrasound irradiation. A central composite design (CCRD) was conducted to evaluate the effects of temperature and power in the range of 30-80 °C and 0-60%, respectively. The determination of enzyme activity was performed. The enzymatic activity was estimated in terms of activity, through the oxidation reaction of the substrate to tetraguaiacol characterized by the orange color. One unit of peroxidase activity was defined as the protein mass that can cause an increase of 0.001 in absorbance unit per minute. The results showed that after treatment with ultrasound using a power of 30% and a temperature of 55 °C was resulted an increase in relative activity, 129.5% in the enzyme from rice bran, 147.9% in the enzyme from soybean meal and 102.4% in the enzyme from horseradish. Using microwave radiation, the highest relative activity (107.4%) was observed for the peroxidase extracted from rice bran with 10 seconds of reaction time and a reaction temperature of about 50 °C. The data obtained in this study suggested that the ultrasonic bath and microwave are adequate for conducting reactions catalyzed by

peroxidases because it was possible to increase their activity during the performed tests. Preliminary tests of enzyme application showed that the highest color removal occurred using 3 mL of the enzyme extract from rice bran and H2O2 concentrations of 40 mg L-1. This condition resulted in a color removal of about 40%.

The Effect of Soybean Meal Heating Time on the in vitro Digestibility and Ruminal Fermentation Profile was analyzed by **Wulandari** *et al.* (2020). The aim of this study was to determine the most optimal heating time in protection of protein rich feedstuff on digestibility and in vitro ruminal fermentation profile. Proteinous feedstuff used in this research is soybean meal (Glycine max). This study is designed using one way ANOVA, with five treatments of heating time (T0 (control)= unheated, T1= 10 min, T2= 20 min, T3= 30 min, and T4= 40 min) at 120 ˚C and 6 replications. All the treatment samples then incubated for 48 h according to the 2-stage in vitro technique. The results showed that protecting soybean meal through heating decreased the dry matter (DM), organic matter (OM) digestibility, NH3 concentration and acetic acid:propionate ratio (A:P) ($p < 0.05$) compared with the control group. In general, there were no significant effects on ruminal pH, total and proportion of volatile fatty acids (VFA), and microbial protein. A decrease in NH3 concentration and A:P ratio was seen in T2 (49.05 mg/100 mL and 1.52, respectively). It can be concluded that protein protection in soybean meal through heat treatment can decrease rumen degradation. The best heating time for protecting soybean meal was found at 20 minutes.

The Effect of partial replacement of soybean and corn with raw or processed chickpea was studied by **Sengül** *et al*,(2020). This research was subjected to investigate the effects of using raw and treated chickpeas of various methods and levels in rations of egg-laying quail on egg yolk content, egg yolk fatty acid profile and some blood related factors. Chickpeas of various treatments including raw, autoclaved, and microwave treated were introduced in the diet along with main course on two different levels of 20% and 40% along with main course. The treatments were planned as seven groups, consisting of control, autoclaved, and microwave treated of both 20% and 40%. Every group was repeated for thrice. Quail

were housed in multi-stored cages for nine weeks. The result showed significant differences between the control and treatment groups in terms of the of linoleic acid, a-linoleic acid, total saturated fatty acids, and palmitic acid content in the egg yolk fatty acids and also exhibit significant in the ALT levels of the blood parameters for the other parameters not significant to that extent.

The research on optimization of producing oil and meal from canola seeds by using microwave- pulsed electric field treatment was studied by **Mohseni** *et al*, (2019). In this study, microwave-pulsed electric field was used for optimization and extraction of canola seeds oil. The microwave treatment varies in times from 0 to 200 s and PEF intensities 0 to 5 kV/cm. The oil was then extracted using screw press with different speeds of 11 to 57 rpm. Oil extraction efficiency, refractive index, peroxide phenolic compounds and refractive index of oil and protein meal were determined. Tocopherols content of the finest sample was also measured. The results expressed that with increase in intensity, time and speed peroxide and phenolic percentage also increased. The efficiency of oil extraction from seeds and protein meal was increased with increase in the MW time and PEF intensity to some extent but then decreased. The efficiency of oil extraction and protein decreased at higher speeds. The refractive index of all samples was 1.475. Gamma tocopherol was predominate one in canola oil and applying the pretreatment led to an increase in the number of total tocopherols. Treating at 1.28 kV/cm and 28.71 rpm for 140.5 s was suggested as the optimum condition with high desirability of 0.744.

The Extraction and Antioxidant Activity of Soybean Saponins from Lowtemperature Soybean Meal by microwave treatment combined with enzymatic hydrolysis (MTEH) was studied by **Liu Zhong-hua** *et al.* (2014). The research aimed at developing an optimal procedure for the extraction of soybean saponins from lowtemperature soybean meal with MTEH, and studied the antioxidant activity of soybean saponins. The result shows that the optimal parameters of microwave treatment, determined with the orthogonal array design method, were the medium fire of microwave power, 1.5 min of microwave time, 80% of ethanol, and 1:25 ratio of material to water, moreover, the optimal conditions of enzymatic hydrolysis,

determined with the response surface experiments, were 50 minutes of hydrolysis time, 51°C of hydrolysis temperature and 1.5% dosage of cellulase, with which the optimal extraction ratio of the soybean saponins reached 0.916%. The saponins extracted from soybean meal exhibited antioxidant activity and the effect of scavenging superoxide anion radicals (SAR) and hydrogen peroxide (HO).

The research on heat processing of soybean kernel and their effect on lysine availability and protein solubility was done by **Sladana**, *et al.* (2006). Soybean kernels of varieties Bosa and ZPS 015 were used in the research. The content of available lysine and proteins were analysed in fresh soybean kernels, soybean products made after the processes of dry extrusion, micronisation, microwave toasting and autoclaving. The technologicalprocedure were used to expose kernals to different temperatures from 57 to 150◦C. The duration of exposure of the soybean kernels to the variying temperature ranged from 25-30 seconds in dry extrusion and 30 minutes in autoclaving. To change chemical composition; i.e. nutritive quality kernels were subjected different sources of heat, causing different thermodynamic processes. The protein content decreased under the influence of higher temperatures in the course of all treatments of processing. The solubility was dropped drastically affected by temperatures of 100◦C in dry extrusion in other treatments it decreases gradually. The content of available lysine was determined with DNFB. The processes of micronisation and microwave treatment showed the greatest effect on the reduction of lysine availability. Dry extrusion and autoclaving, performed within closed systems – in which the increased moisture content has a special effect – resulted in significantly slighter changes of the available lysine content.

The research on the effect of enzymatic and microwave treatment on the Indian defatted rice bran was carried out by **Bandyopadhyay** *et al.* (2012). The main aim of research was to analyze protein content. To enhance the protein solubility by physical means performs various attempts such as microwave digestion as well as by microwave digestion followed by homogenization. Compared to 1 Min conventional boiling which recovers only 28.9% protein, the microwave treatment for 40 sec recovers 78.4%. Even protein recovery is further increased in microwave

treatment followed by homogenization. Defatted rice bran meal has also been subjected to enzymatic treatment using enzymes such as papain and viscozyme separately to increase the protein solubility. The yield of protein isolate (RPI), prepared by alkaline extraction followed by acidic precipitation is 10.2% and by papain and viscozyme treatment increased to 14.5 & 22.4% , 21.1 & 22.3% by microwave treatment and microwave treatment followed by homogenization respectively. A maximum of 82.5 and 82.6% protein has been recovered as soluble protein from de-oiled bran by viscozyme treatment and by 40 sec microwave treatment followed by 10 min of homogenization. The results demonstrate for the recovery of protein from defatted rice bran microwave treatment followed by homogenization is finest method.

The research on effect of microwave treatment on rice bran and i were studied by **Pokkanta** *et al*.(2022). The research mainly focused to determine phytochemical content and antioxidant activity. The rice bran was subjected to microwave treatment with varying in time and wattage. The treatment from 130 to 880 W is used and time varies from 0.5-5.0 min. Through UV-Vis spectrometry method antioxidant activity, total flavonoids, and total phenolic contents were determined. Different phytochemicals such as Tocols, γ-oryzanols, squalene, phytosterols and phenolic compounds were quantified by using high-performance liquid chromatography. The results expressed that antioxidant activity were increased by 0.5 times, total phenolic contents by 1.3 times, total flavonoid contents by 0.9 times, total tocols by 2.6 times, total γ-oryzanols by 1.6 times, and total phytosterols by 1.4 times. Phytochemicals such as trans-p-coumaric acid and kaempferol enhanced by 10.3 times and 8.6 times respectively. This research exposed that for stabilization of rice brand and to increase the phytochemical utility microwave treatment is finest procedure. For several industries it serves as potential method for using rice bran as feed ingredient. The microwave treatment at 440 W for 2.5 min is more effective and also provides the best contents of the bioactive compounds and antioxidant activity.

The research work to improve the shelf- life of rice bran by domestic heat treatments was carried out by Ahmed, *et al*. (2007). In the experiment the treated material was provided as feed to rats for assessment of dietary consumption. Three different varieties of rice bran were used and subjected to heat treatment procedures such as pan roasting, pan roasting with oil and microwave roasting to enhance the shelf life. At the end of a 3-month storage period there is vast difference in free fatty acid content and it was 12 to 23-fold in the absence of heat treatment and in heat treatment it was 1.6 to 2.5- fold. In an animal trail, to determine safety of consumption and palatability pan-roasted rice bran was fed to rats at 5% and 10% levels. In 10% rice bran group the mean food intake was slightly higher compared to control i.e. 13.57 ± 0.2 g in 10% group and 12.31 ± 0.52 g in control. Compared to control rats feed with 10% rice bran gained higher body weight which attributes higher food consumption by these animals. Dietary rice bran did not show any adverse effect on the hematological parameters. Serum albumin level increased in rice bran-fed groups and remaining serum protein level was similar to that of the control group except, which is indicative of liver sufficiency in rice bran treatment. The weights of vital body organs such as liver, kidney, heart and testes were not affected by rice bran either at the 5% or 10% level. It is inferred that simple domestic heat treatments such as pan roasting and microwave heating can effectively check the rancidity in rice bran for a considerable period of storage, making it suitable for animal consumption.

The research on Microwave stabilization and process optimization of rice bran cultivar Jhelum variety was done by **Asad**, et al. (2021). The research mainly focused on optimizing microwave stabilization process of rice bran obtained from freshly milled paddy (Jhelum variety) using response surface methodology. This study was conducted at different moisture contents ranging from 12% to 30%, sample weight from 5–250 g, and heating time ranging from 1–5 min which was examined to determine their influence on parameters such as free fatty acids (FFAs) and color coordinates. The microwave treatment shows effective influence on these parameters which studied by regression analysis. Stabilization treatments resulted in prevention of hydrolytic rancidity by reduction of FFAs, prime indicator of lipase

activity, thereby making it fit for human consumption. The results indicate that 16% sample moisture, 100 g sample weight, and 4 min microwave heating time are optimum conditions for microwave stabilization. Through microwave treatment Proximate analysis showed that due to microwave treatment crude protein and ash contents increased while crude fat and crude fiber contents reduced in rice bran.

The research in fermentation of defatted rice bran and gamma-amino butyric acid (GABA) synthesis by lactobacillus was done by **Tuan**, *et al*. (2017). This research focused on the synthesis of GABA by Lactobacillus bacteria in fermentation devoid of adding glutamic acid. Two strains of Lactobacillus i.e. VTCC - B - 454 and VTCC - B – 890 were investigated into capacity of GABA synthesis. Result indicates that compared to Lactobacillus plantarum VTCC - B – 890 variety Lactobacillus brevis VTCC - B - 454 exhibits higher capacity of GABA synthesis in fermentation of defatted rice bran extract than. Significant decrease in Total dissolved solid (TDS), free amino acids (AA) and reducing sugar (RS) contents in fermentation of defatted rice bran extract with two strains also observed. Lactobacillus brevis VTCC - B - 454 accumulates 2,952 ppm of GABA in at pH 5 and 9 %w/w of TDS in 24 hours of fermentation in defatted rice bran extract. The result implies that fermentation with Lactobacillus brevis VTCC - B - 454 can be applied for GABA production from defatted rice bran extract than VTCC - B – 890.

The research on Phenolic profile and antioxidant activity of fermented heat-stabilized defatted rice bran (HDRB) were studied by **Webber**, *et al*. (2014). HDRB serves as a potential source of phenolic compounds. An estimated 70% of phenolics present in rice bran are esterified to the arabinoxylan residues of the cell walls. Release of such compounds could provide a value-added application for HDRB. The objective of this study was extraction and quantification of phenolic compounds from HDRB by using fermentation technology. The total of 8 organisms selected for rice bran fermentation, the bacteria Bacillus subtilis subspecies subtilis had the maximum phenolic release of 26.8 mg/ gm HDRB as ferulic acid equivalents (FAE). Further release of rice bran phenolics were optimized by response surface methodology. At 168 h, 0.01% inoculation level, and 100 mg HDRB/mL an

optimum of 28.6 mg FAE/g rice bran was predicted. Phenolic concentration and radical scavenging capacity significantly increased in fermentation of HDRB for 96 h with B. subtilis subspecies subtilis. Fermented rice bran had 1.38 mg caffeic acid, 6.03 mg syringic acid, 19.02 mg (-)-epicatechin, 4.64 mg ferulic acid, 4.86 mg gentistic acid, 10.04 mg sinapic acid, , 4.08 mg p-courmaric acid and 17.59 mg benzoic acid per 100 g fermented extract compared to 0.65 mg p-courmaric acid and 0.36 mg ferulic acid per 100 g non-fermented extract. The high phenolic content and antioxidant activity of fermented HDRB extract indicates that rice bran fermentation under optimized condition is more effective compared to non-fermented HDRB.

The research on comparative analysis of Total Phenolic, Antioxidant Activity, γ-Oryzanol and β-Glucan content in fermented rice bran of various varieties was studied by **Jung**, *et al*. (2017). Rice bran is a rich source of bioactive compounds. Recent studies have suggested that the biological activites of rice bran improved by fermentation. This study focused to determine the level of Total Phenolic, Antioxidant Activity, γ-Oryzanol and β-Glucan of fermented rice bran from 21 different Korean varieties. They also assessed determining γ-oryzanol content in fermented rice brans by analytical method. Among the fermented rice brans, the Haedam rice bran variety contained the maximum level of total phenol content of 156.08 mg GA equivalents/g, DPPH radical scavenging activity of 71.30% and ORAC (Oxygen radical absorbance capacity) value of 1101.31 μM trolox equivalents/g. Foreover, the highest level of γ-oryzanol content of 294.77 ± 6.74 mg/100 g was showed by the fermented Migwan rice bran variety.

The research work on in-vitro fermentation patterns of rice bran components by microbiota was studied by **Pham**, *et al*. (2017). Polyphenols (RBPP) and Soluble feruloylated arabinoxylan oligosaccharides (FAXO) isolated from rice bran are hypothesized to have positive impacts on human gut microbiota through a prebiotic function. FAXO and RBPP treatments were assessed for short-chain fatty acids (SCFA) production patterns and by evaluating their impacts on the phylogentic composition of human gut microbiota by 16S rRNA gene sequencing. Fresh fecal samples collected from healthy adults (n = 10, 5 males, 5 females) were diluted with

anaerobic medium. Each sample received five treatments: CTRL (no substrates), FOS (fructooligosaccharides), FAXO, RBPP, and MIX (FAXO with RBPP). Samples were incubated at 37 °C and an aliquot was withdrawn at 0, 4, 8, 12, and 24 h. Results showed that SCFA production was significantly increased with FAXO and was comparable to fermentation with FOS. RBPP did not increase SCFA productions, and no significant differences in total SCFA production were observed between FAXO and MIX, indicates that RBPP does not modify FAXO fermentation. Changes in microbiota population were found in FAXO treatment, especially in Bacteroides, Prevotella, and Dorea populations, which indicates that FAXO might modulate microbiota profiles. RBPP and MIX increased Faecalibacterium, especifically F. prausnitzii. Combined FAXO and RBPP fermentation increased abundance of butyrogenic bacteria, Coprococcus and Roseburia, suggesting some interactive activity. Results expressed that the potential for FAXO and RBPP from rice bran to promote colon health through a prebiotic function.

The Effects of concentrated and dephytinized wheat bran and rice bran addition on bread properties were determined by **Özkaya** B *et al*. (2017). Wheat bran and rice bran were concentrated in terms of dietary fiber and were dephytinized by two different methods (fermentation and hydrothermal). Following treatments such as Untreated, concentrated, concentrated-dephytinized by fermentation method and concentrated-dephytinized by hydrothermal method were used. Each bran samples were incorporated into flour at levels of 0, 10, 15, and 20%, and their effects on bread properties were determined. Unprocessed wheat bran and rice bran addition decreased the volume yield, and the specific volume of the bread depends on the incorporation level. However, the dephytinization treatments slightly improved these values. The same pattern was observed for the total number of cells and the total cell area of bread crumbs. Both wheat bran and rice bran were observed to cause a darker crumb color, and the effects of bran samples on crumb color were more pronounced after the dephytinization treatment. The addition of concentrated wheat bran and rice bran significantly increased hardness, as well as decreasing springiness, cohesiveness, and resilience of the bread, depending on the bran levels.

Although dephytinization treatments enhanced the textural properties of bread, these results were still inferior to those obtained using bread produced with untreated bran. The results showed that dephytinization treatments, the influence of fermentation treatment on bread properties was slightly beneficial compared to hydrothermal treatment.

The research work on effect of microwave treatment and analysis of physiochemical properties was studied by **Roman**, *et al.* (2015). The research mainly focused on analyzing micro-structure and physiochemical properties. The maize flour was subjected to microwave frequency of 400W of different time interval of 0.5, 1, 2 and 4 Min. Results expressed that microwave subjected maize flour has less compact structure and more swallow starch granules. At 0.5 and 1 min treatment maize flour shows high viscosity and shows breakdown in structure compared to untreated. The maize flour subjected to high time range of 2 and 4 min displays low viscosity and breakdown. Generally microwave treatment creates changes in starch crystalline structure and amylose-lipid complexes which effects functional and thermal properties of flour.

The research on killing silo insect pest *sitophilus zeamais* motsch in maize through microwave treatment was studied by Hartulistiyoso (2013). The research mainly focused on mortality of *sitophilus zeamais* and analysis of starch and protein content due to microwave treatment. The dry maize of 200 gms was subjected to different microwave frequencies of 240, 480 and 720 W respectively for 60, 90 and 120 s. The treatment of 720W for 60 s found more effective compared to hot air oven and aso100% mortality achieved. The short-time microwave treatment maintains 68.5% starch and 9.4% protein and kills pest totally.

The research on microwave treatment and its effect on reduction of Aflatoxin B1and Ochratoxin A in maize flour were studied by **Alkadi and Altal** (2019). The research mainly focused on reduction of Aflatoxin B1 and Ochratoxin A in artificially contaminated maize flour. The maize flour was subjected to microwave frequency of 2450 Hz for 0, 2, 4, 6, 8 and 10 min. The results suggested that effective reduction of both the toxins Aflatoxin B1 and Ochratoxin A concentration

with increase in time period of treatment. At 0 min the concentration of Aflatoxin B1 and Ochratoxin A is 100%, after 4 min treatment concentration reduced by 25% and after 10 min treatment the concentration almost reduced by 75%.

The influence of microwave treatment on the structural and functional properties of pure amylose and amylopectin of corn was studied by **Zhong** *et al*. (2021). Both pure granular amylose (AM) and pure granular amylopectin (AP) granules have high nutritive value. The research mainly focused on effect of microwave treatment on corn starch and to analyze the structural and functional properties. The granules are subjected to microwave frequency of 400 W of various time range of 1-8 min. The results expressed that first three min treatment decreases the molecular weight of both the AM and AP granules. Initially crystalline structure of AM starch increased from 15.6% to 20.6% and later it decreased to 11.3%. In contrast to AM starch the crystalline structure of AP starch initially decreased from 18.9% to 10.8% and later increase to 20.0%. Long-range microwave treatment to AM granules significantly increases resistant starch and water solubility.

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Chapter 3

MATERIAL AND METHODS

The whole research was carried out in Biochemistry and Animal Nutrition laboratories, FVAS, IAS, RGSC, BHU, Barkaccha, Mirzapur, India.

3.1 Experimental Material

The feed stuffs Maize, soybean meal and de-oiled rice bran which were used for the research work was collected from Livestock Food Complex (LFC) situated in FVAS, IAS, RGSC, BHU, Barkaccha, Mirzapur, India. Two replicates for each feed of same variety used for research.

3.2 Microwave Treatment

The Feed stuffs were subjected to microwaves which differ in frequency and time. Totally 5 different treatments are given to each replicate including control. Initially the feed was weighed and 20gms of feed sample is used for each treatment. Pulse treatment is given to the feed by adding water at regular intervals to avoid charring. The frequency and time range of treatment to feed stuff is mentioned in following tables

Table 7: Soybean Meal (SBM) microwave treatment

S.No	Treatment type	Frequency	Time
1	Control	0	0
2	Treated 1	300 watts	5 min
3	Undertreated 1	300 watts	2.5 min
4	Treated 2	450 watts	3 min
5	Undertreated 2	450 watts	1.5 min

Table 8: De-oiled Rice Bran (DORB) and Maize grains microwave treatment

S.No	Treatment type	Frequency	Time
1	Control	0	0
2	Treated 1	800 watts	3 min
3	Undertreated 1	800 watts	1.5 min
4	Treated 2	600 watts	4 min
5	Undertreated 2	600 watts	2 min

3.3 Proximate analysis

The major chemical constituents of soybean meal including moisture, ash, crude fat, crude fiber and crude protein were determined in duplicate.

3.3.1 Instruments

a. Aluminium moisture cup with lid

b. Hot Air Oven

c. Metal Tongs

d. Desiccator

e. Analytical Balance

f. Analytical Balance

g. Vapodest 50s Protein Analyser and digester(turbo therm)

h. Gerhardt digestion Tube

i. Volumetric Flask

j. Beaker

k. Glass filter crucible with fused sintered glass filter plate/Fibre bag pore size 40-90 μm.

l. Cylinder of at least 270 ml with a reflux condenser, suitable for boiling.

m. Extraction apparatus

n. Extraction thimbles, free of matter soluble in light petroleum and having porosity consistent with the requirements

o. Muffle furnace

p. Hot plate

q. Meker burner

r. Desiccator

s. Funnel

t. Whatman filter paper No. 42

u. Conical flask

3.3.2 Reagents used

a. Standard Solutions 0.1N HCl:

b. H_3BO_3 (2%): Dissolve 20 g of Boric acid in 1000ml D/W.

c. Concentrated H_2SO_4 (98%)

d. NaOH (40%) solution: Dissolve 40 g of Sodium hydroxide pellet in 50mL D/W and make up to 100 ml D/W.

e. Catalyst Mixture / tablet: This is used to induce oxidation and also to raise the boiling point without any spurting. The catalyst mixture is composed of 3.5 g of sodium /potassium sulphate + 0.4 g $CuSO_4$.

f. pH Solutions (pH 4 & 7)

g. Sodium Carbonate (99%) Certified Reference Material (CRM)

h. Ammonium Sulfate (99%)(CRM)

i. Potassium / Sodium sulfate (GR grade)

j. Copper Sulfate (GR Grade)

k. Sulphuric acid = 1.25 % solution (c = 0.13 mol/l).

l. Anti-foaming agent (e.g. n-octanol).

m. Filter aid (Celite 545 for crucibles).

n. Light petroleum boiling-range 40˚ to 60˚C.

o. Potassium hydroxide solution= 1.25% solution (c = 0.23 mol/l).

p. Dilute Hydrochloric Acid- approximately 5 N. prepared from concentrated hydrochloric acid.

q. Silver nitrate solution

3.3.3 Determination of Moisture

Principle: Dry matter was determined gravimetrically as the residue remaining after drying at 100 ºC in a hot air oven.

Procedure

1. Dry aluminium cup in oven at 100ºC for 15 to 30 minutes. Cool in a desiccator (not mere than 20 min), weigh and record M weight.

2. Accurately weighed about 5 g of the prepared sample (Grind about 200 g-300g of the sample. Passed it through the 1.00-mm Sieve in the grinding mill. The sample should be of homogenous and uniform particle size. Transfer the grinded sample in proper packet to keep it moisture free) in a tared aluminum dish with a cover, having a diameter of at least 50 mm and a depth of about 40 mm and record M1 weight.

3. Place the cup with sample in hot air oven maintained at 100 ± 2ºC and dry for at least 2 h. Cooled in a desiccator and weigh (M2).

4. The process of heating for 30 min was repeated, cooling and weighing until the difference between two successive weighing was less than one mg.

Calculation

Moisture (%) =100(M1-M2)/M1-M

Where,

M1= mass in g of the dish with the material before drying,

M2= mass in g of the dish with the material after drying,

M= mass in g of the empty dish.

3.3.4 Determination of Protein

Principle: Protein determination in sample was undertaken by Kjeldahl method. Method has three step procedures. (I) Digestion, (II) Distillation, (III) Titration.

$$\text{Protein} \xrightarrow[\text{Heat, Catalyst}]{\text{Sulfuric Acid}} (NH_4)_2SO_4$$

$$(NH_4)_2SO_4 + 2NaOH \longrightarrow 2NH_3 + Na_2SO_4 + 2H_2O$$

$$NH_3 + H_3BO_3 \text{ (boric acid)} \longrightarrow NH_4 + H_2BO_3^{-(\text{Borate ion})}$$

$$H_2BO_3^- + H^+ \longrightarrow H_3BO_3$$

Ammonia nitrogen was quantified by titration with a standard 0.1 N HCl solution. After calculating the nitrogen content of the sample, crude protein content is calculated by multiplying with respective protein factor. e.g. for feed 6.25.

A reagent blank was carried through the analysis and the volume of HCl titrant required for this blank is subtracted from each determination.

Procedure

A. Preparation of the Digest:

a. About 0.5 - 1.0 g of the sample transferred into a clean dry digestion tube (approx. 1.0 g for material with 3% to 30% protein and approx. 0.5 g for 30% to 80% protein)

b. Then, carefully add about 20 ml of Concentrated H_2SO_4 (A.R.-98%) in the tube through the sides. Gently rotate the tube to mix well the sample and the acid.

c. About 8-10 g catalyst mixture or 2 tablets was added to the content of the flask.

d. Place the digestion tubes in electric digestion unit of Turbo therm.

B. Digestion

When working with a Turbotherm-System with 250 ml Kjeldatherm-digestion tubes, the following program parameter was recommended:

After completing the program keep the tube till fumeless and cooled.

Steps	**Time in min.**	**Temperature ºC**
1	0 – 20	0-210
2	15	210-410
3	60	420
4	10	Cooling

C. Distillation and Titration:

a. The distillation was carried out on Vapodest protein analyser which is automated distillation and titration system. The system has facility to automatically add of water, NaOH, boric Acid and removal of content by tubes through suction after analysis.

b. The digestion tubes were shaken and mixed well before making use of the digest for distillation. The digested samples were loaded in the Vapodest carousel. Operate the samples were analyzed through the software as per working instruction of Vapodest Protein Analyser. Automatically instrument started adding NaOH, H_2O with steam and the ammonia gets distilled into the Boric acid. After collection of all ammonia in the boric acid titrations being with 0.1N HCl. Results were calculated by using the software based upon the amount used of 0.1 N HCl consumed.

Calculation:

$$\% \text{ Protein} = 1.4007 \times T \times F \frac{(\text{Consumption (ml)} - \text{Blank value (ml)})}{\text{Weight of the sample (g)}}$$

Where,

T: Normality of titrant

F: Factor i.e. 6.25 for feed.

Expression of Result:

Crude Protein in g/100 g or (%)

3.3.5 DETERMINATION OF CRUDE FIBER

Principle

The sample, defatted where necessary, was treated successively with boiling solutions of sulphuric acid and potassium hydroxide of specified concentrations. The residue was separated by filtration on a sintered-glass filter washed, dried, weighed and

ashed within a range of 495˚C to 500˚C. The loss of weight resulting from ashing corresponds to the crude fiber present in the test sample.

Procedure

1. one gram of prepared sample was weighed and placed (M_1) in the crucible/fiber bag.

2. The crucible and fiber bags were placed in crucible/fiber bag in de-fatting assembly for the de-fatting of samples.

3. Assembled the heating unit and the filter crucible/fiber bag, and then cylinder was attached to the crucible. About 150 ml of boiling sulphuric acid was poured into the assembled cylinder and crucible and if necessary added a few drops of anti-foaming agent. The liquid was brought to the boil within 5 ± 2 minutes and vigorously boiled for exactly 30 minutes.

4. the tap was opened to the discharge pipe and, under vacuum, the sulphuric acid was filteredthrough the filter crucible/fiber bags and washed the residue three times consecutively with boiling water, ensuring that the residue was filtered dry after each washing.

5. Closed the outlet tap and poured 150 ml boiling potassium hydroxide solution to the assembled cylinder and crucible and added a few drops of anti-foaming agent. The liquid was brought to the boil within 5 ± 2 minutes and vigorously boiled for exactly 30 minutes.

6. Filter and repeated the washing procedure used for the sulphuric acid step. After the final washing and drying, disconnected the crucible/fiber bags and its contents.

7. Dryed the crucible/fiber bags in the oven at 130˚C nearly 2 hours. After drying, cooled the crucible in desiccator and weighed rapidly (M_2).

8. Placed the crucible in a muffle furnace and ashed to constant weight (loss in weight between two successive weightings must be less than or equal to 2 mg) at 495˚C to 500˚C for at least 30 minutes (M_3).

9. After each heating cooled first in the furnace and then in the desiccator before weighing.

Calculation of results

The crude fiber content as a percentage of the sample was given by the expression:

Crude Fiber = $(M_3-M_2)/M_1 \times 100$

Where:

M_1 = weight of sample (in g)

M_2 = crucible weight after drying (in g)

M_3 = weight of crucible after ashing (in g)

3.3.6 DETERMINATION OF CRUDE OILS AND FATS

Principle

The sample was extracted with light petroleum benzene. The solvent was distilled off and the residue dried and weighed.

Procedure

1. 1 g of the sample weighed (M_1), transfered it to an extraction thimble and covered with a fat-free cotton wool.

2. Took the weight of Empty cup (M_2).

3. Placed the thimble in extraction cup and then placed the cup in extractor and poured 150 ml of petroleum benzene. Ran the set programme (mentioned below) for 2hours and 30 minutes. Collected the light petroleum extract in a dry, weighed flask containing fragments of pumice stone/ glass beads.

Extraction temperature	150˚C
Hot extraction	50 min
Evaporation	4×interval
Extraction	1h 10min
Evaporation B	4 × interval
Evaporation C	5 min
Program length	2h 29min

4. Placed the cups in hot air oven for one and a half hour at 100˚C±2

5. Left to cool in a desiccator and weighed. Dryed again for 30 minutes to ensure that the weight of the cup remained constant (loss in weight between two successive weighing must be less than or equal to 1 mg).

Expression of result

Expressed the weight of the residue as a percentage of the sample.

Calculation,

Crude fat = $[M_3\text{-}M_2/M_1] \times 100$

Where, M_3 = weight of cup after drying in grams

M_2 = weight of empty cup in grams

M_1 = sample weight in grams.

3.3.7 DETERMINATION OF TOTAL ASH

1. Principle

Ash is the inorganic residue left after ignition of a decarbonized material in a muffle furnance at 550 °C for 2 hours.

Procedure

1. Took the weight of porcelain, silica or platinum dish or crucible (M).

2. Weighed accurately about 2 g of the dried material (M_1) in a tared porcelain, silica or platinum dish.

3. Ignited with the flame of a Meker burner or hot plate for about half an hour (chearing).

4. Completed the ignition by keeping in a muffle furnace at 550 ± 20°C (2 hours) until grey ash results, cooled in a desiccator and weighed.

5. Ignited the dish again in the muffle furnace for 30 minutes, cooled in desiccator and weighed (M_2).

6. Repeated this process until the difference in mass between two successive weighing is less than 1 mg.

7. Noted the lowest mass and used in calculation.

8. Took this sample for AIA, if required.

Calculation

Total ash (As such basis), percent by mass= $(M_2\text{-}M) \times 100/M_1$

Where, M_2 = the lowest mass in g of the dish with the ash,

M = mass in g of the empty dish, and

M_1 = mass in g of the sample.

3.3.8 DETERMINATION OF ACID INSOLUBLE ASH

Procedure

1. Took the weight of porcelain, silica or platinum dish (M).

2. Weighed accurately 2 g of the dried material (M_1) in a tared porcelain, silica

or platinum dish.

3. Ignited with a Meker burner for about one hour. Completed the ignition by keeping in a muffle furnace at 550 0 ± 20°C (2 hour) until grey ash results or take sample from Total Ash as outline at 2.7 in TA.

4. Moisten with concentrated hydrochloric acid and evaporate to dryness.

5. Keep in an electric air-oven maintained at 135 ± 2°C for about 3 hours or keep it on hot plate at 200°C for 2 hour.

6. Add 25 ml of dilute hydrochloric acid and heat until level become half, Cooled and filter through Whatman filter paper No. 42 or its equivalent.

7. Washed the residue with hot water until washings are free from chlorides as tested with silver nitrate solution and Return the filter paper and residue to the dish.

8. Ignited it in a muffle furnace at 550° ± 20°0 for one hour. Cooled in a desiccator and weigh.

9. Ignited the dish again for 30 minutes (M_2), cool and weigh, repeated this process till the difference between two successive weighing is less than one milligram.

Calculation

Acid insoluble ash, percent by mass= $(M_2-M)/M_1 \times 100$

Where,

M_2 = the lowest mass in g of the dish with the acid insoluble ash,

M = mass in g of the empty dish, and

M_1 = mass in g of sample.

3.4 Biochemical and Anti-oxidant analysis

3.4.1 Reagents required

a. Methanol – 70%

b. plant extract sample

c. Folin–Ciocalteu reagent (FC reagent)

d. Sodium carbonate (Na_2Co_3)

e. Distilled water

f. Gallic Acid

g. Sodium Nitrite ($NaNo_2$)

h. Aluminium chloride ($AlCl_3$)

i. Sodium hydroxide (NaOH)

j. DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate)

k. Quercetin

l. Ascorbic Acid

m. Sulphuric acid (H_2So_4)

n. Sodium phosphate

o. Ammonium molybdate

3.4.2 Instruments used

a. Coffee grinder

b. Magnetic stirrer

c. Conical flask

d. Sieve Mesh (40 mm)

e. Whatman filter paper No 1

f. Funnel

g. Spectrophotometer

h. Hot water bath

3.4.3 Sample preparation for biochemical and anti-oxidant analysis

The plant extract was finely grinded by using coffee grinder and passed through 40 mm mesh and collected. Each plant sample of 1gm dissolved in 25 ml of 70% methanol in a conical flask. This solution was stirred continuously by using magnetic stirrer for minimum14 hrs. After 14 hrs the solution was filtered by using Whatman filter paper No1. The filtrate was collected in 50 ml centrifuge tube. After filtration volume of each filtered sample was adjusted to15 ml by adding 70% methanol. The samples are stored at -20^0C for future use.

3.4.4 Determination of total phenolic content

The total phenolic content in feed ingredients was determined by using Folin–Ciocalteu procedure (Bamdad et al. 2006). In test tube 1ml of plant extract was mixed with 5 ml of FC reagent (10 times diluted). Then add 7.5% of 4 ml sodium carbonate (Na_2Co_3). The solution was mixed well and allowed to stand for 30min. absorption was measured by using spectrophotometer at wavelength of 760 nm. Gallic acid used as standard.

3.4.5 Determination of total flavonoid content

Total flavonoid content was determined spectrophotometrically by using $AlCl_3$ reagents. In a test tube 1 ml of plant extract was and 4 ml of distilled water was added. There after 0.3 ml of 5% $NaNO_2$ was added and vortexed. Then incubate for 5 min. After incubation 10% of 0.3 ml $AlCl_3$ was added and incubated again for 6

min. After 6 min 2 ml of 1M NaOH and 2.4 ml distilled water was added. Then absorbance was measured at 510nm using spectrophotometer. Rutin was used as standard.

3.5 Determination of antioxidant property by invitro method

3.5.1 DPPH free radical scavenging activity

Antioxidant activity of the soybean meal was determined by using DPPH (1,1-diphenyl-1-picrylhydrazyl). This procedure described by Mohdaly et al. (2011). Initially 22 mg of DPPH was dissolved in 70% methanol (1×10^{-3} M) and stock solution prepared and stored at -20^0C for further use. The 6 ml of stock solution was dissolved in 100 ml 70% methanol to get absorbance value of 0.8 at 517 nm for working solution (6×10^{-5} M). The plant extract of 50 µL was mixed with 5 ml of DPPH working solution. It was incubated in dark for 30 Min and absorbance was measured at 517 nm using spectrophotometer. The ascorbic acid was used as standard (std conc 2.5, 5, 7.5, 10, 15, 20, 30, 40 and 60 µg/ml). The scavenging effect was determined by using formula

Scavenging activities (%): $[(A_c - A_s) / A_c] \times 100\%$

3.5.2 Total antioxidant capacity

The total antioxidant capacity of soybean meal was determined by phosphomolybdate assay procedure described by Prieto et al. (1999). Initially the reagent solution (0.6 M sulphuric acid, 28mM sodium phosphate, 4mM ammonium molybdate) was prepared. Plant extract of 0.1ml was mixed with 1ml of reagent solution in micro-centrifuge tube. The centrifuge tubes were capped tightly and incubated in water-bath at 95^0C for 95 min. After incubation the samples were allowed to cool at room temperature. Then absorbance was measured at 765 nm against blank using spectrophotometer. Ascorbic acid used as standard (conc 12.5, 25, 50, 100, 200 and 400 µg /ml) and calibration curve was plotted.

Total Antioxidant activity (%): $[(A_c - A_s) / A_c] \times 100\%$

3.6 SDS-PAGE analysis

3.6.1 Sample preparation for SDS-PAGE analysis

The sample of about 150 mg were weighed and taken in micro centrifuge tube. The defatting solution (chloroform: methanol: acetone- 2:1:1) of 1ml added to the tube and tubes are vortexed. The tubes were incubated at room temperature for minimum 3 hrs. After incubation the supernant carefully decanted without disturbing the pellets. The samples were dried, after drying the extraction buffer (5% 2-β-marceptomethanol, 2% SDS, 8 M urea, 0.0625 M tris-HCl pH-6.8) of 0.5 ml was added to each tube and incubated at room temperature for overnight. Next day tubes were centrifuged at 10000 RPM for 20 Min. After centrifugation supernant collected in separate centrifuge tubes and store the collected samples at-20^0C for SDS-PAGE analysis.

Table 9: Reagents for SDS – PAGE Electrophoresis *

1.	**Resolving Gel**	**12.5 % to make 6 ml**
	Distilled water	1.893 ml
	30% / 0.8% Acrylamide / Bis acrylamide	2.501 ml
	tris-HCL buffer (1.5 M pH 8.8)	1.5 ml
	10% SDS**	60 µl
	Ammonium persulphate	45 µl
	TEMED	3 µl
2.	**stacking Gel**	**4 % to make 2.5 ml**
	Distilled water	1.5 ml
	30% / 0.8% Acrylamide / Bis acrylamide	0.335 ml
	Tris-HCL buffer (1.5 M pH 6.8)	0.625 ml
	10% SDS	150 µl
	Ammonium persulphate ***	12.5 µl

	TEMED	2.5 µl
3.	**Gel Loading Bffer**	**5 % to make 15 ml**
	10% SDS**	4 ml
	10 mM β-Mercaptoethenol	0.8 ml
	20 % Glycerol	2 ml
	0.2 M tris-HCl Buffer (pH 6.8)	300 µl
4.	**Electrode Buffer**	
	0.05 M Tris 12 g	
	0.192 M Glycine 28.8 g	pH 8.2-8.4
	0.1% SDS 2 g	No adjustment
	Water to 2 L	Required
	May be used 2-3 times	
5.	**Protein Staining Solution*** **	
	0.1 % Coomassie brilliant blue R 250	0.1 g
	40% Methanol	40 mL
	10% Acetic acid	10 mL
	Distilled water	50 mL
	First, dissolve the dye in methanol and Proceed.	
	Destainer	As above without the Dye

* Stored at 4^0C.

** Stored at room temperature.

*** Freshly prepared for each time of gel casting.

3.6.2 Procedure for setting electrophoresis

1. Assembled the plates for casting gel.

2. The separating gel was poured to a height of 3cm below the lower plate and allowed to polymerize.

3. After the gel is set, the comb was mounted on the top of the glass plate after stacking gel solution was poured to the top of the glass plate and allowed to set.

4. After 20 minutes the comb was carefully removed.

5. The wells were loaded with protein samples and marker.

6. The entire assembly was then mounted on the electrophoretic equipment and both bottom reservoirs and top reservoirs were made filled with the electrode buffer.

7. Filling of buffer into the top reservoirs was done very carefully so that the samples loaded into the wells remain un-agitated.

8. The electrophoretic assembly (BIO-RAD Mini Protena Tetra System) was connected to power supply with red lead to anode and black to cathode.

9. Constant voltage of 100 volts was applied in electrophoresis till the dye front travelled up to the bottom of the plate.

10. The power was turned off and gel plate assembly detached gently from the equipment.

11. The gel plates were carefully removed using a spatula in a plastic tray filled with water. The gel was carefully lifted with the support of a polythene sheet and placed onto the bottom of the staining tank and the stain solution poured into the tank with the lid covered on the top of the tank. The gel was left for staining for 5 hours with intermittent shacking. After which the stain solution was removed and replaced with distaining solution and left overnight for distaining.

12. The next day the distaining solution was removed and the gel was washed with distilled water and image has been captured.

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Chapter 4

RESULT AND DISCUSSION

3.4.1 Proximate Analysis

The research mainly focused on increasing the digestibility of the conventional feed stuffs by protein degradation using the microwave treatment without affecting the biochemical and anti-oxidant properties. Three different feed stuffs Maize grains, Soybean Meal (SBM), De-Oiled Rice Bran (DORB) are treated using microwave oven of varying frequency and time.

The results of proximate analysis does not show any significant changes in all the three feeds compared to control. The dry matter content was slightly increased due to microwave heating. The crude protein content in the feed increased slightly due to covalent bond breakage in protein. The remaining parameters fat content, total ash, AIA and crude fiber content remain almost same without any significant changes.

The SBM which is protein rich meal has average protein content of 54-57% and after microwave heating crude protein content slightly increased and AIA content slightly decreased from 3 to 1.9, the fat content in treated 1 (300W for 5 Min) decreased slightly and the crude fiber content is about 54-57%. Overall there is not any significant change in the treated samples compared to control. So it is evident that microwave treatment doesn't show any adverse on the major chemical constituents of SBM.

Table10: Proximate analysis of SBM in %

Chemical Properties	Control	Treated 1	Under treated 1	Treated 2	Under treated 2
Dry Matter *	93.73±0.21	93.31±1.58	91.91±0.05	96.85±0.29	96.23±0.43
Total Ash	9.974±0.54	9.363±0.35	10.85±0.74	9.72±0.22	9.813±0.73
AIA	3.36±0.08	1.967±0.06	2.593±0.14	2.47±0.19	2.887±0.14
Ether Extract	2.207±0.10	1.487±0.01	2.23±0.18	2.14±0.10	2.48±0.24
Crude Protein	55.73±0.05	60.12±0.47	56.23±0.27	54.8±2.09	55.14±0.04
Crude Fiber	54.97±2.03	57.42±0.98	55.95±1.11	55.88±1.58	54.76±1.02

(For each quality parameter, common letters row-wise across indicate the lack of a significant difference ($P > 0.05$) based on ANOVA).

*dry matter content decrease in treated samples due to sprinkling of water during treatment to avoid charring

DORB is the bran rich in fiber content and used as a feed supplement to animals. The quality of the feed is determined by the AIA content. Increase in AIA content results decrease in quality of the feed. The AIA content of the DORB varies from 4.9 to 5.3 indicating the microwave treatment doesn't affect the AIA content. It observed that slight increase in crude protein content of DORB after subjected to microwave frequency. The microwave treatment shows more effect in feeds with more fiber content. After the treatment crude fiber content in both treated 1(800W for 3Min) and under-treated 1(800W for 1Min 30 sec) increased slightly indicating that microwave frequency of 800W showing mere effect on crude fiber content in DORB. The crude fiber content in DORB varies from 12.5 to 17%. As the DORB is already de-oiled the fat content is very low and there is no any significant change in fat content before and after the treatment. The dry matter and ash content in DORB range from 85 to 90% and 11 to 13% respectively. There is not any significant

changes both in dry matter content and ash content indicating that no adverse effect in feed. The results indicated that microwave treatment to the DORB doesn't show any changes in major chemical constituents of the feed.

Table11: Proximate analysis of DORB in %

Chemical Properties	**Control**	**Treated 1**	**Under treated 1**	**Treated 2**	**Under treated 2**
Dry Matter	89.97±0.92	85.87±3.93	81.94±2.06	91.27±1.36	84.17±4.70
Total Ash	11.77±0.07	12.83±0.06	13.53±0.05	13.07±0.06	13.89±0.01
AIA	4.99±0.03	4.43±0.05	5.07±0.10	4.817±0.08	5.33±0.25
Ether Extract	0.97±0.026	0.65±0.011	0.88±0.003	0.84±0.02	0.94±0.01
Crude Protein	14.79±0.05	16.21±0.09	16.28±0.40	16.62±0.33	15.44±0.68
Crude Fiber	13.13±0.39	17.14±0.84	16.96±0.24	12.64±0.05	15.23±0.63

(For each quality parameter, common letters row-wise across the feed indicate the lack of a significant difference ($P > 0.05$) based on ANOVA).

Maize grains which are reach source of carbohydrates provide energy in the diet of both humans and livestock. The microwave treatment in maize doesn't show any adverse affect. The crude protein content in maize slightly increased after the treatment. The crude protein in maize varies from 7 to 9%. There is no effect in fat content which remains almost same before and after treatment. There is slight decrease in crude fiber content from 15 to 9.5% but there is not showing any significant change. The AIA content decreased slightly in the treated samples compared to control. The remaining properties dry matter and ash content also doesn't show any significant changes. It evident that microwave treatment in maize doesn't affect major chemical constituents. There is no any adverse affect in maize due to microwave treatment.

Table12: Proximate analysis of Maize in %

Chemical Properties	control	Treated 1	Under treated 1	Treated 2	Under treated 2
Dry Matter	90.69±0.27	77.96±2.53	78.08±2.25	81.71±0.69	88.63±2.17
Total Ash	2.073±0.05	1.033±0.05	1.7±0.15	1.467±0.07	1.953±0.15
AIA	2.66±0.18	1.62±0.03	1.88±0.04	1.82±0.05	2.28±0.10
Ether Extract	2.783±0.08	3.007±0.01	3.357±0.21	3.067±0.03	2.917±0.09
Crude Protein	7.784±0.01	9.927±0.28	9.149±0.28	9.05±0.30	9.097±0.25
Crude Fiber	15.25±0.34	9.15±0.05	9.387±0.13	12.37±0.4	11.91±0.52

(For each quality parameter, common letters row-wise across both lentil types indicate the lack of a significant difference (P > 0.05) based on ANOVA and Tukey‘s HSD).

3.4.2 Biochemical Analysis

The biochemical analysis includes determination of Total Phenolic Content (TPC), Total Flavonoid Content (TFC), DPPH free radical scavenging activity and Total anti-oxidant activity (TAOA). The effect of microwave treatment on feed stuffs related to these properties were analyzed. The results expressed, there was no adverse effect of microwave treatment on feed stuffs.

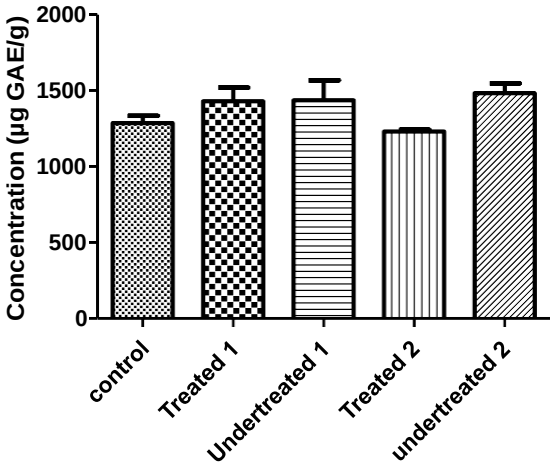
Table13: Biochemical and Anti-oxidant activity of Feed stuffs

	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH (mg AAE/g)	TAOA (mg QE/g)
Maize				
Control	1.29±0.05	1.08±0.18	1.15±0.09	3.75±0.07
Treated 1	1.43±0.89	1.07±0.21	1.63±0.26	3.92±0.23
Undertreated 1	1.43±0.13	1.07±0.14	1.26±0.13	3.33±0.27
Treated 2	1.23±0.01	0.99±0.02	1.01±0.09	3.03±0.11
undertreated 2	1.48±0.06	0.98±0.06	1.42±0.12	3.96±0.16
SBM				
Control	2.59±0.27	2.14±0.35	2.59±0.38	9.81±1.45
Treated 1	3.17±0.54	2.12±0.36	4.11±1.01	12.62±2.13
Undertreated 1	3.31±0.27	2.20±0.15	3.65±0.78	13.31±1.67
Treated 2	3.35±0.30	2.23±0.23	3.87±0.75	13.17±1.44
undertreated 2	3.36±0.38	2.49±0.21	3.54±1.24	12.39±1.61
DORB				
Control	2.35±0.24	3.82±0.39	2.01±0.02	6.95±0.38
Treated 1	2.18±0.10	3.53±0.13	3.04±0.22	7.88±0.22
Undertreated 1	2.48±0.33	3.26±0.55	2.93±0.33	8.47±1.03
Treated 2	2.63±0.02	3.41±0.01	2.68±0.12	9.66±0.69
undertreated 2	2.66±0.11	3.47±0.21	2.85±0.35	9.57±1.17

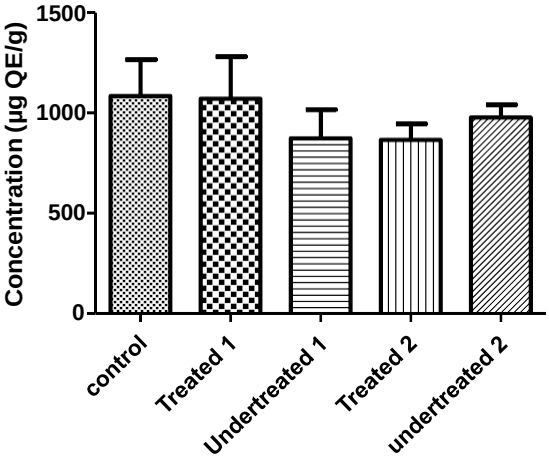
(For each quality parameter, common letters row-wise across the feed indicate the lack of a significant difference ($P > 0.05$) based on ANOVA).

Note: TPC- Total Phenolic Content, TFC- Total Flavonoid Content, DPPH- DPPH free radical scavenging activity, TAOA- Total Anti-Oxidant Activity.

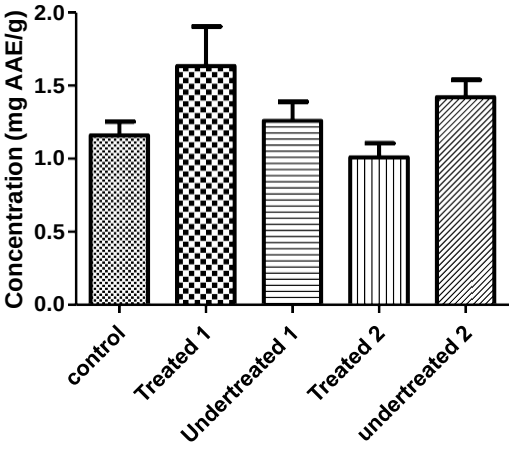
Graph1:Total Phenolic Content in Maize



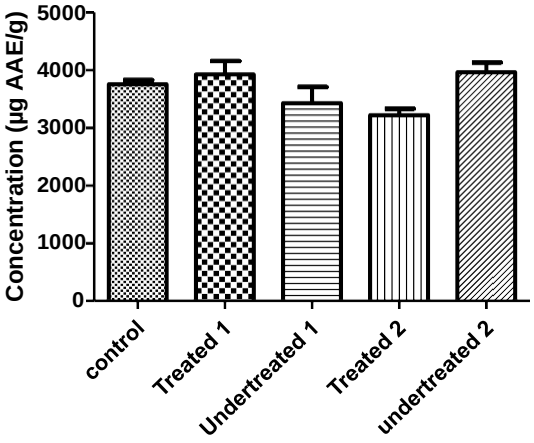
Graph2: Total Flavonoid Content in Maize



Graph3: DPPH Scavenging Activity

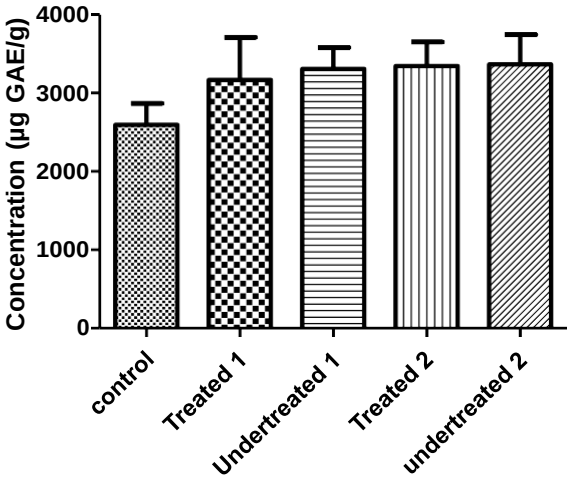


Graph4: Total Anti-Oxidant Activity in Maize

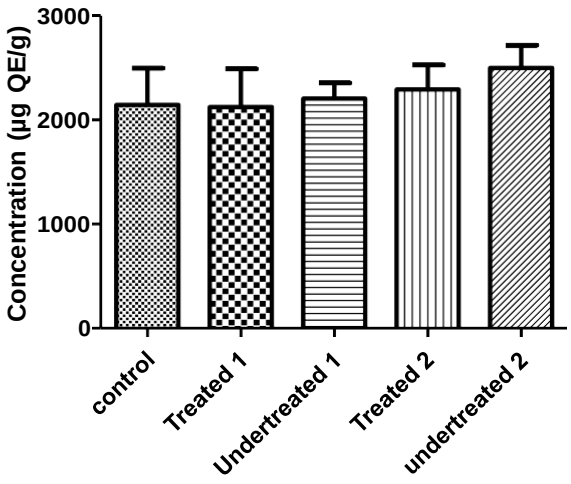


The effect of microwave treatment in maize and the biochemical and anti-oxidant properties were determined and the results indicates there is no significant changes in both phenolic content and flavonoid content. The results was expressed in Gallic acid equivalent (GAE) for phenolic content and for flavonoid content expressed in Quercetin equivalent. The average phenolic content in the maize was about 1.29 to 1.49 mg GAE/g dry weight, the flavonoid content was about 0.9 to 1.1mg QE/g dry weight. So it evident that both biochemical properties of the maize doesn't affected by the microwave treatment. The anti-oxidant properties DPPH free radical scavenging activity as well as total anti-oxidant activity was analyzed. The results of both the anti-oxidant properties expressed in Ascorbic acid equivalent (AAE) and the results indicates that treated 1and undertreated 2 increase slightly compared to control. The treated 1 sample shows about 1.63 mg AAE/g and the Undertreated 2 sample 1.42 mg AAE/g where as control shows 1.15 mg AAE/g. The analysis of total anti-oxidant activity results shows almost same results. The Treated 1 and undertreated 2 shows slightly more activity compared to control. The treated 1sample shows about 3.96 mg AAE/g dry weight and Undertreated 2 shows 3.92 mg AAE/g dry weight. The control expresses almost same activity of 3.75 mg AAE/g dry weight. There was no Significant changes observed in all the properties which indicated no adverse effects of microwave treatment on maize.

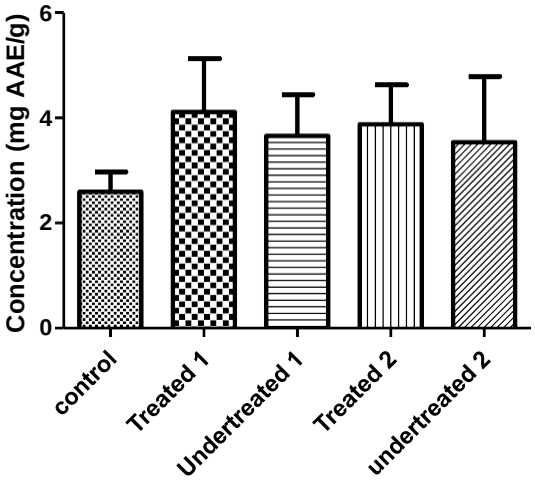
Graph5: Total Phenolic Content in SBM



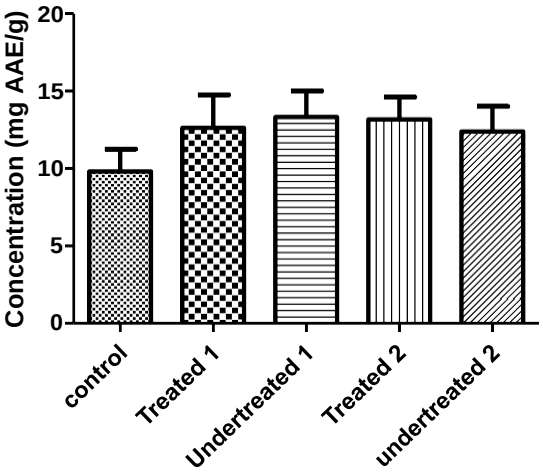
Graph6: Total Flavonoid Content in SBM



Graph7: DPPH Scavenging Activity

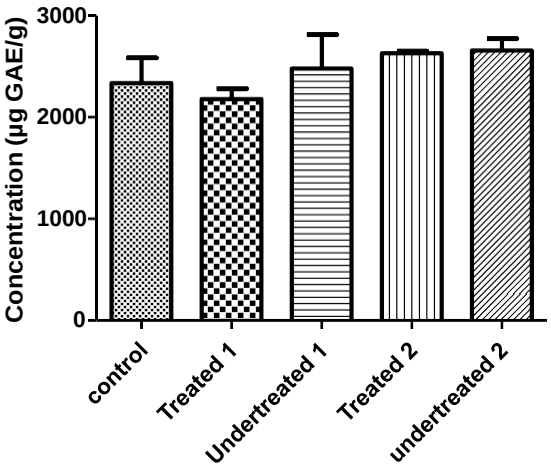


Graph8: Total Anti-Oxidant Activity in SBM

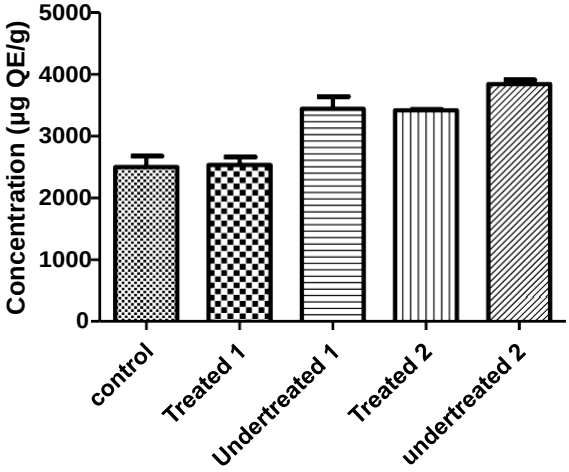


The effect of microwave treatment in SBM was analyzed and the four parameters doesn't show any significant affect compared to control. The TPC content slightly increased in treated SBM samples in related to control. The TPC content of control was about 2.59 mg GAE/g dry weight and for the treated samples it varies from 3.1 to 3.4 mg GAE/g dry weight. Slight increase in TPC content indicates that microwave treatment doesn't affect or reduce the phenolic constituents in the feed content. The flavonoid content also doesn't show any significant changes in the SBM due to microwave treatment. The flavonoid content in SBM varies from 2.1 to 2.5 mg QE/g dry weight. The highest flavonoid content was observed in undertreated 2 i.e. 2.49±0.21 mg QE/g dry weight where as control has 2.14±0.35 mg QE/g dry weight. The anti-oxidant properties DPPH scavenging activity and total anti-oxidant activity were analyzed. The results showed that DPPH scavenging activity increased slightly the highest scavenging activity was shown by treated1 which was about 4.11±1.01 mg AAE/g dry weight and whereas in control scavenging activity was about 2.59±0.38 mg AAE/g dry weight. It was evident that due to microwave treatment scavenging activity slightly increased but insignificant. The TAOA was also increased slightly in treated samples compared to control. The TAOA content in control SBM was about 9.21 mg AAE/g dry weight and the treated SBM varies from 12 to 13 mg AAE/g dry weight. The results shown that due to microwave treatment in SBM slight increase in biochemical and anti-oxidant properties was observed and didn't show any adverse effect.

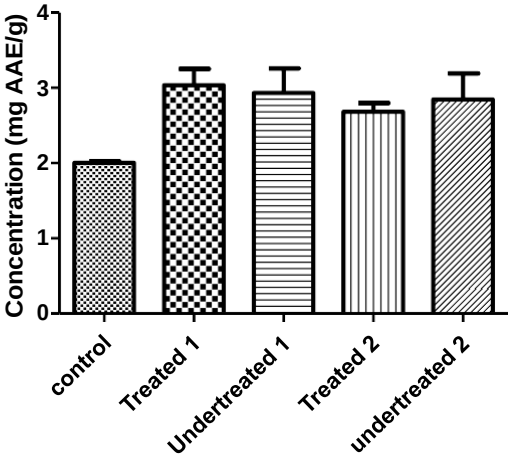
Graph9: Total Phenolic Content in DORB



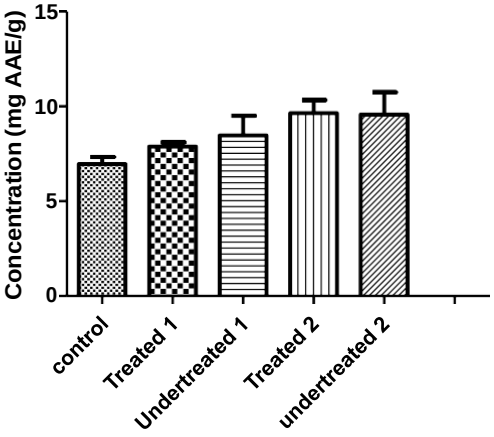
Graph 10: Total Flavonoid Content in DORB



Graph11: DPPH Scavenging Activity



Graph12: Total Anti-Oxidant Activity in DORB



The effect of microwave treatment in DORB was analyzed and the results show that TPC and TFC content not affected significantly. The average TPC content of DORB was about 2.3 to 2.6 mg GAE/ g dry weight. The average TFC content was about 3.2 to 3.8 mg QE/g dry weight. So, it is evident that there is not any significant change in the TPC and TFC due to microwave treatment. As earlier mentioned DORB rich in fiber content, there is little bit changes in both anti-oxidant properties. The DPPH scavenging activity and TAOA of DORB slightly increased due to effect of microwave treatment. The DPPH scavenging activity of treated DORB samples was about 2.6 to 3.1 mg AAE/g dry weight, whereas in control it was about 2.01 mg AAE/g dry weight. The TAOA of the DORB slightly increased in treated samples was about 7.9 to 9.5 01 mg AAE/g dry weight. The TAOA in control was about 6.95 mg AAE/g dry weight. The microwave treatment didn't show any significant changes or any adverse effects on the DORB.

3.4.3 SDS PAGE Analysis

The present investigation on SDS-PAGE was carried out on three different conventional feed ingredients to analyze the effect of microwave frequency on protein subunits of the feed stuffs. The three feed stuffs are different in their protein content in which SBM very rich in protein and DORB rich in fiber and Maize rich in carbohydrate content. The aim experiment was to analyze the amount of protein degradation in microwave treated samples as compared to control. In the present investigation, the total soluble proteins (Tris-HCl soluble) were extracted from cotyledons of the seeds following the procedure of Mukhlesure and Hirata (2004). Loading wells with 10 µL of protein extract revealed sharper bands.

Sodium Dodecyl Sulphate – Polyacrylamide Gel Electrophoresis (SDS–PAGE) as described by Laemmli (1970) is a powerful and dependable technique for characterization of proteins. In principle, it involves extraction of proteins from the target materials, such as feed stuffs, straw, bran, cotyledons, leaves etc, followed by its denaturation into polypeptides in the presence of SDS and β-mercaptoethenol that breaks disulphide bonds. SDS has mainly two functions: it denatures secondary, tertiary and quaternary structures by binding to hydrophobic protein sites and then its binding confers a net negative charge on the resulting denatured proteins or the polypeptide. The protein subunits are then separated through a gel (polyacrylamide) in an electric field according to their molecular weights.

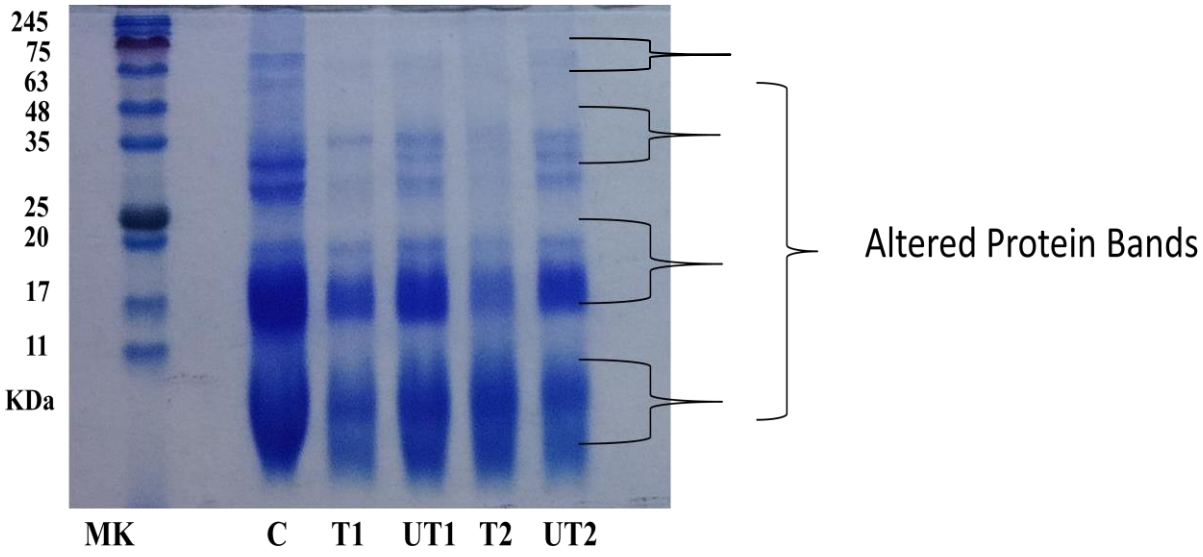


Figure1: SDS-PAGE analysis of Maize

(Line1- Molecular Marker (MK), Line2- control (C), Line3- Treated1 (T1), Line4- Under-treated1 (UT1), Line5- Treated2 (T2), Line6- Under-treated2 (UT2))

The SDS-PAGE analysis of Maize showed that the protein bands in treated samples degraded and at the same range the bands appeared clearly in control. Mainly at the molecular marker weight at 25 to 35 KDa the bands were clearly degraded in both treated1 and treated2 and the bands in under-treated1 and under-treated2 degraded slightly. The bands in control almost clearly appeared. The same trend was observed the protein bands at molecular weight of 17 to 20 KDa. The bands were degraded in SDS-PAGE but the crude protein content slightly increased. The degraded protein bands indicated that protein digestibility maybe increased in treated Maize samples (Figure1).

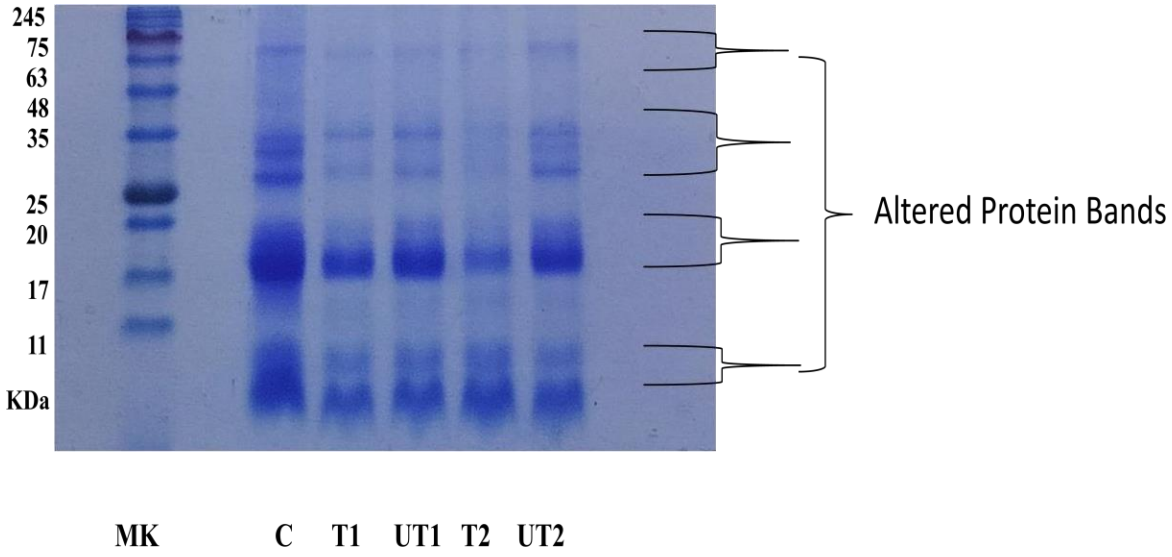


Figure2: SDS-PAGE analysis of DORB

(Line1- Molecular Marker (MK), Line2- control (C), Line3- Treated1 (T1), Line4- Under-treated1 (UT1), Line5- Treated2 (T2), Line6- Under-treated2 (UT2))

The SDS-PAGE analysis in DORB showed clear difference between control and treated samples. In DORB the protein brands degraded in the treated samples while in control the bands appeared more clearly. The protein bands at molecular weight of 75 KDa disappeared in treated1 and treated2, in Undertreated1 and Undertreated2 the bands were a bit lighter whereas in control the bands were comparatively thicker. The bands between 25 to 35 KDa almost disappeared in all the treated samples and in control it was slightly visible. The same trend was observed at the molecular weight of 20 and 11 KDa. The difference observed in protein bands revealed that protein degradation was increasesd in treated samples due to the effect of microwave treatment (Figure2).

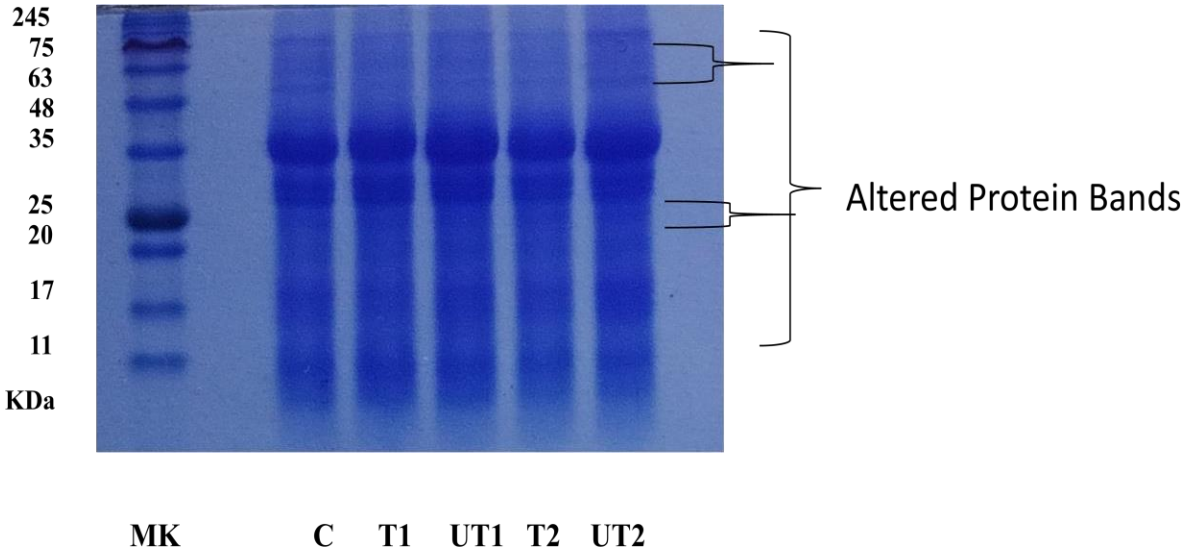


Figure3: SDS-PAGE analysis of SBM

(Line1- Molecular Marker (MK), Line2- control (C), Line3- Treated1 (T1), Line4- Under-treated1 (UT1), Line5- Treated2 (T2), Line6- Under-treated2 (UT2))

The SDS-PAGE analysis in SBM was done to differentiate amount of protein degradation in sample. The protein bands in the treated samples of the SBM were lighter compared to the control. Particularly bands at molecular weight of 63 KDa the bands in treated samples got smeared and lighter whereas in control it was a bit darker. The same pattern was observed in bands at molecular weight of 25 KDa. The result indicated that the protein degradation might be there in the treated bands, which was not observed in control (Figure3).

Chapter-5

SUMMARY AND CONCLUSION

The research work was done to determine the effect of microwave treatment on the conventional feed ingredients. The main aim was to improve the digestibility of feed ingredients or to replace the conventional feed ingredients with unconventional feed stuffs. This helps in minimizing the cost of feed ingredients which is major issue in feed industry present days. The feed ingredients are treated with microwave frequency of varying time and frequency range. The research also focused on developing the optimum conditions of microwave treatment for different feed stuffs. The effect of microwave treatment was analyzed by different tests such as proximate analysis, biochemical analysis, Anti-oxidant activity analysis and SDS-PAGE analysis. The degradation in protein bands in SDS-PAGE analysis indicated the structure of protein i.e. quaternary, tertiary, and secondary structures are denatured and converted into basic amino acids which is major factor that defining the increase in protein digestibility. The overall crude protein not decreased even after the treatment. By analyzing the two parameters it is proven that microwave treatment to the conventional feed stuffs increases the protein digestibility. The analysis of properties such as TPC, TFC, DPPH scavenging activity and TAOA which doesn't shows any significant changes it proven that there is no adverse affects on the feed stuffs because of microwave treatment. So microwave treatment to feed stuffs maybe recommended with optimum time and frequency range. The time and frequency of treatment to the feed stuffs was varies depending on type, color, texture and moisture content of the feed. Generally oil cakes need frequency about 300 to 450W and for the bran the higher frequency of 800W for short time is needed. Care should be taken while giving treatment to the feed that incase of high frequency and time sometimes treated materials may get charred and leads to reduction in feed quality.

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REFERENCES

Şengü, A. Y., & Çalişlar, S. (2020). Effect of partial replacement of soybean and corn with raw or processed chickpea. *South African Journal of Animal Science*, *50*(3), 461-470.

Ahmed, F., Platel, K., Vishwanatha, S., Puttaraj, S., & Srinivasan, K. (2007). Improved shelf- life of rice bran by domestic heat processing and assessment of its dietary consumption in experimental rats. *Journal of the Science of Food and Agriculture*, *87*(1), 60-67.

Alkadi, H., & Altal, J. (2019). Effect of microwave oven processing treatments on reduction of Aflatoxin B1 and Ochratoxin A in maize flour. *European Journal of Chemistry*, 10(3), 224-227.

Asad, S., Jabeen, A., Aga, M. B., Majid, D., Jan, N., Amin, T., & Mehraj, F. (2021). Microwave stabilization and process optimization of rice bran cultivar Jhelum. *Journal of Food Processing and Preservation*, *45*(7), e15659.

Tangendjaja, B. (2022). Quality control of feed ingredients for aquaculture. In *Feed and feeding practices in aquaculture* (pp. 165-194). Woodhead Publishing.

Bandyopadhyay, K., Chakraborty, C., & Barman, A.K. (2012) Effect of microwave and enzymatictreatment on the recovery of protein from Indian defatted rice bran meal. *Journal of Oleo Science*,61(10):525-529.

Pantalone, V. R. (2012). Modern breeding approaches to enhancing soy protein quality. In *Designing soybeans for 21st century markets* (pp. 197-226). AOCS Press.

Caprita, A., & Caprita, R. (2010). Modification of the soluble protein content of heat-processed soybean flour. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *38*(2), 98-101.

Dat, L. Q., Ngan, T. T. K., & Nu, N. T. X. (2017, September). Gamma-amino butyric acid (GABA) synthesis of Lactobacillus in fermentation of defatted rice bran extract. In *AIP Conference Proceedings*,1878 (1), 020045.

Wang, F., Sukmanov, V., Zeng, J., & Jiang, J. (2020). Improving the quality of soybean by-products by physical methods during its use in bakery technology. Review. *Ukrainian Food Journal*, *9*(3).

Golshan, S., Pirmohammadi, R., & Khalilvandi-Behroozyar, H. (2019). Microwave irradiation of whole soybeans in ruminant nutrition: Protein and carbohydrate metabolism in vitro and in situ. In *Veterinary Research Forum*,10 (4), 343.

Hartulistiyoso, E. (2012). Microwave treatment for killing the silo insect pest Sitophilus zeamais Motsch in maize. In *II Asia Pacific Symposium on Postharvest Research Education and Extension: APS2012 1011*, 293-298.

Jung, T. D., Shin, G. H., Kim, J. M., Choi, S. I., Lee, J. H., Lee, S. J., ... & Lee, O. H. (2017). Comparative analysis of γ-oryzanol, β-glucan, total phenolic content and antioxidant activity in fermented rice bran of different varieties. *Nutrients*, *9*(6), 571.

Chashnidel, Y., Tymuri Yanesari, A., & Mohajer, M. (2019). Evaluation of poultry byproduct meal as a substitute for soybean meal on growing performance, blood metabolites and carcass characteristics of lambs. *Journal of Ruminant Research*, *7*(1), 1-18.

Kumar, R., & Rao, P. S. (2019). Effect of Different Thermal and Non-Thermal Treatments on Total Phenols and Antioxidant Content of Soybean. In *International Journal of Recent Technology and Engineering* (IJRTE), 8(4), 1512–1515.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *nature*, *227*(5259), 680-685.

Zhong-Hua, L., Hong-Lian, G., Rui-Ling, L., & Jin-Hui, Z. (2015). Extraction and antioxidant activity of soybean saponins from Lowtemperature soybean meal by MTEH. *The Open Biotechnology Journal*, *9*(1).

Meshram, S., Deo, A. D., Kumar, S., Aklakur, M., & Sahu, N. P. (2018). Replacement of de- oiled rice bran by soaked and fermented sweet potato leaf meal: Effect on growth performance, body composition and expression of insulin- like growth factor 1 in Labeo rohita (Hamilton), fingerlings. *Aquaculture Research*, *49*(8), 2741-2750.

Szabó, G., Rajkó, R., Kovács, E., & Vidal-Valverde, C. (1997, November). Optimisation of microwave treatment for reducing enzyme activity of soya bean. In *International Symposium on Applications of Modelling as InnovativeTechnique in the Agri-Food Chain. MODEL-IT 476* (pp. 141-148).

Nahavandinejad, M., Seidavi, A., Asadpour, L., & Payan-Carreira, R. (2014). Blood biochemical parameters of broilers fed differently thermal processed soybean meal. *Revista MVZ Córdoba*, *19*(3), 4301-4315.

Mohseni, N. M., Mirzaei, H. O., & Moghimi, M. (2020). Optimization of producing oil and meal from canola seeds using microwave− pulsed electric field pretreatment. *OCL*, *27*, 2.

Özkaya, B., Baumgartner, B., & Özkaya, H. (2018). Effects of concentrated and dephytinized wheat bran and rice bran addition on bread properties. *Journal of texture studies*, *49*(1), 84-93.

Pham, T., Savary, B. J., Teoh, K., Chen, M. H., McClung, A., & Lee, S. O. (2017). In vitro fermentation patterns of rice bran components by human gut microbiota. *Nutrients*, *9*(11), 1237.

Pokkanta, P., Yuenyong, J., Mahatheeranont, S., Jiamyangyuen, S., & Sookwong, P. (2022). Microwave treatment of rice bran and its effect on phytochemical content and antioxidant activity. *Scientific reports*, *12*(1), 1-7.

Rezaii, F., & Bayat Kohsar, J. (2019). Effects of microwaving, and moist and dry heating on ruminal degradability of protein and dry matter in soybean

meal. *Journal of Livestock Science and Technologies*, *7*(1), 11-19.

Román, L., Martínez, M. M., Rosell, C. M., & Gómez, M. (2015). Effect of microwave treatment on physicochemical properties of maize flour. *Food and Bioprocess Technology*, *8*(6), 1330-1335.

Roslan, M. A. M., Sobri, Z. M., Zuan, A. T. K., Cheak, S. C., & Rahman, N. A. A. (2022). Bioprospecting microwave-alkaline hydrolysate cocktail of defatted soybean meal and jackfruit peel biomass as carrier additive of molasses-alginate-bead biofertilizer. *Scientific reports*, *12*(1), 1-14.

Golunski, S. M., Scapini, T., Modkovski, T. A., Marques, C. T., Camargo, A. F., Preczeski, K. P., ... & Treichel, H. (2017). Commercial and noncommercial peroxidases activity under ultrasound and microwave treatment: a pretreatment to improve wastewater treatment. *Journal of the Brazilian Chemical Society*, *28*, 1890-1895.

Afifi, M., & Alkaladi, A. (2014). Antioxidant system in Uromastyx philbyi during hibernation and activity periods. *Central European Journal of Biology*, *9*(9), 864-868.

Banaszkiewicz, T. (2011). Nutritional value of soybean meal. *Soybean and nutrition*, *12*, 1-20.

Pankaj, S. K., Wan, Z., & Keener, K. M. (2018). Effects of cold plasma on food quality: A review. *Foods*, *7*(1), 4.

Webber, D. M., Hettiarachchy, N. S., Li, R., Horax, R., & Theivendran, S. (2014). Phenolic profile and antioxidant activity of extracts prepared from fermented heat- stabilized defatted rice bran. *Journal of Food Science*, *79*(11), H2383-H2391.

Wulandari, W., Widyobroto, B. P., Noviandi, C. T., & Agus, A. (2020). The Effect of Soybean Meal Heating Time on the in vitro Digestibility and Ruminal Fermentation Profile. *Iranian Journal of Applied Animal Science*, *10*(4), 595-601.

YOSHIDA, H., & KAJIMOTO, G. (1988). Effects of microwave treatment on the trypsin inhibitor and molecular species of triglycerides in soybeans. *Journal of food science*, *53*(6), 1756-1760.

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