NUTRIENT AND ANTINUTRIENT COMPOSITIONS OF EXTRUDATES FROM FERMENTED AND SPROUTED RICE-SESAME BLENDS

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MTech/SLS/2018/8627

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ABSTRACT

In Nigeria, directly extruded products have grown very popular, particularly among children. However, the majority of these foods have minimal nutritional value and considered poor providers of protein and vital nutrients due to the utilization of sole cereal in their production. This study evaluated the nutrient and antinutrient contents of extrudates produced from fermented and sprouted rice-sesame flour blends. Rice and sesame grains were each fermented and sprouted and the sesame was defatted using hydraulic press. Five (5) composites each were developed with sesame flours at 10, 20, 30, 40 and 50% from fermented and sprouted rice-sesame flours and evaluated for proximate composition. The moisture contents of the blends were adjusted to 25 % moisture levels and extruded using a laboratory single-screw extruder where it plasticizes and form extrudates that could be used for functional properties determination. The extrudates were subjected to proximate composition, minerals, some B-vitamins (B_1, B_2, B_6 and B_{12}) and amino acid profile determination. The antinutrient (tannin, oxalate and phytate) contents of the extrudates were also determined. Extrusion cooking resulted in the reduction of protein (from the range of 10.26 - 24.39 % to 7.26 - 17.46 % and 11.60 - 25.35 % to 8.28 - 20.35 %) in the fermented and sprouted seed blends respectively. The fat contents also reduced (from the range of 6.92 - 15.42 % to 2.04 - 6.30 and 4.51 - 11.52 % to 1.06 - 4.60 %) in the fermented and sprouted seed blends respectively, while the ash, fibre and carbohydrate significantly increased in all the extrudates when compared with their flour blends. Extrudates produced from fermented and sprouted rice-sesame seed flours at substitution level of 50/50 % gave significantly higher concentrations of Vitamin B_1 (0.93 and 0.88 mg/100g), Vitamin B_2 (0.47 and 0.79 mg/100g), Vitamin B_6 (0.27 and 0.66 mg/100g), and Vitamin B_{12} (1.11 and 0.58 mg/100g) respectively. Similarly, the mineral (Na, K, Ca, Mg, P, Fe) contents increased with sesame addition in the extrudate samples except Zn where extrudates produced from fermented and sprouted rice - sesame seed flours at substitution level of 60/40 % gave significantly higher values (0.94 and 1.70 mg/100g) respectively. The limiting amino acid (lysine) was highest in $FR_{60}S_{40}$ (5.59 ± 0.64 g/100g) and $SR_{50}S_{50}$ (5.23 ± 0.19 g/100g) respectively. Extrudates of the sprouted seed blends had lower phytate levels and higher oxalate contents when compared to their fermented seed counterparts. The functional properties significantly improved with sesame addition in both set of treatments while extrudates with 40 % levels of sesame substitutions had higher general acceptability (6.95 ± 1.09 and 6.60 ± 0.50) for the fermented and sprouted extrudate samples respectively. Consequently, the extrudates from fermented and sprouted rice-sesame blends could serve as a source of nutrients for the production of convenient complementary foods to overcome protein-energy-malnutrition among the vulnerable groups.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

The food companies have been forced to use local produce to develop new goods due to the increased concern about eating nutritious meals. In essence, different processing technologies have aided in changing food components into healthier goods with greatest amount of nutritional content to guarantee the population in developing nations has access to enough nutreints (Soycan *et al*., 2019). In the production of novel meals like morning cereal, weaning foods, and other modified starches from cereals, extrusion cooking technique has become more prevalent (Sharma *et al*., 2015). Extrusion cooking technology is a continuous high temperature short time (HTST) food processing method in which mechanical energy is combined with heat energy to create new shapes and textures, as well as to inactivate enzymes, destroy toxins, and lower microbial activity (Harris *et al.,* 2019). Many foods and food ingredients, including breakfast cereals, snack foods, baby foods, pasta products, extruded bread, modified starches beverages, powders, meat and cheese analogues, textured vegetable protein, and blended foods like corn starch and ground meats, have been produced using it for several years in the cereal industry (Kowalczewski *et al*., 2019). It is a technology with high degree of adaptability and efficiency, is inexpensive, produce a lot in a short period of time, reacts quickly, and generates little waste (Gao *et al*., 2019).

Cereal meals like bread and cookies have grown highly popular in Nigeria, particularly among kids. However, the majority of these food have minimal nutritional value with low protein content (Olagunju and Ifesan, 2013). The commonly use cereals are rice, wheat and maize because of their high energy level. However, cereals foods offer great challenges from nutritional point of view based on their swelling power during cooking, the limited availability of their mineral content and the presence of antinutritional factors reduces their bioavailability. In contrast to other cereals, rice and rice-based products are unique because they are easier to digest, hypoallergenic, low in fat, and have a white dazzling hue, all of which are important qualities in the formulation of weaning foods. Additionally, its low fat content makes it ideal for baking for baking. Furthermore, the protein in rice is also rich in sulphur as it contains essential amino acids such as methionine and cysteine, but deficient in lysine (Ufot *et al*., 2018).

The enrichment of cereal-based foods with other protein sources such as oil seeds and legumes have received considerable attention because oil seed and legume proteins are high in lysine, which is an essential amino acid absent in most cereals (FAO, 2001). Sesame seeds (*Sesamum indicum*) are important oil seeds of different diversity (RMRDC, 2004). Sesame seed is a staple food among many ethnic groups in Nigeria and it is cultivated in most areas of the middle belt and some northern states of Nigeria. It contains 44 - 52.5 % oil, protein (18 - 23.5 %) and carbohydrate (13 %). The seeds are consumed fresh, dried or blended with sugar. It is also used as a paste in some local soups (Akusu *et al*., 2019). However, raw sesame seeds contain antinutrients mainly oxalate and phytate usually found in the seed hulls which can adversely affect mineral bioavailability in human nutrition. Enhancement of the nutritional quality of sesame seeds can be anticipated through processing techniques (such as soaking, roasting, fermentation and sprouting) prior to consumption.

Fermentation is one of the oldest known food processing methods and its history stretches back to the Neolithic period, as indicated by archaeological findings of clay tools for cheese making. Its unique ability to enhance the sensory properties of raw materials and preserve the developed product has been recognized throughout human history as miscellaneous fermented products are part of the culinary and cultural heritage of many countries globally (Plé *et al*., 2015). Fermentation is a desirable process of biochemical modification of primary food matrix brought about by microorganisms and their enzymes (Nkhata *et al.,* 2018). It is used to enhance the bioaccessibility and bioavailability of nutrients from different crops and improves organoleptic properties as well as extending the shelf life. It makes food safe by not only inhibiting growth of pathogenic bacteria due to antimicrobial activity of lactic acid, but also detoxifies aflatoxin. Recently, fermentation technology has been brought to the forefront again since it provides a solid background for the development of safe products with unique nutritional and functional attributes (Marco *et al*., 2017). With these desirable benefits, fermentation has been considered as an effective way to reduce the risk of mineral deficiency among populations, especially in developing countries where unrefined cereals and/or pulses are highly consumed (Nkhata *et al.,* 2018).

On the other hand, sprouting is a complex metabolic process during which the lipids, carbohydrates and storage proteins within the seeds are broken down in order to provide energy and amino acids necessary for the plant development. The process has been reported to cause the most significant reduction in antinutrients possibly due to increase in enzymatic activity and bioactive compounds within the seeds. Likewise, the metabolic changes that take place during the different stages of sprouting influence the bio-accessibility of essential nutrients (Akusu *et al*., 2020). Other processing methods such as soaking, cooking and roasting have been reported to improve the nutritional and functional properties of plant seeds. These processing techniques can also reduce malnutrition by making micronutrients available for easy absorption, hence, increasing the utilization of sesame seeds. Industrial processing and utilization of sesame have not been fully developed in Nigeria as its utilization is restricted to producing regions. For most part, the surplus crop is commercialized, bulked and exported with minimal processing limited to cleaning and drying. The present study therefore, is focused on the nutritional and antinutritional composititons of extrudates produced from fermented and sprouted rice-sesame blends.

1.2 Statement of the Research Problem

Directly extruded snacks also referred to as second generation snacks are trendy in the market, but considered products of poor nutritional quality mainly due to the utilization of sole cereals such as rice, wheat, corn and oats in its production (Felix-Medina *et al.*, 2020). It is a highly preferred food product by children, and therefore need to be nutrient dense. However, the daily consumption of this could result in protein-energy-malnutrition in children. In 2016, the World Health Organization (WHO) reported that poor access to nutritionally dense foods including snacks in developing countries in Africa was responsible for half of the death of children under the age of 5 years. In 2015 alone, it was estimated that more than one-quarter of all children under 5 years with wasted muscles lived in Africa. This problem is further aggravated by the menace of epidemic diseases that increased the number of the vulnerable populations, resulting in wide spread of malnutrition in most of the countries (Danbaba *et al*., 2015).

1.3 Aim and Objectives of the Study

The aim of the study is to determine the nutrient (proximate composition, vitamin content, mineral composition, essential amino acid profile) and antinutrient composition (phytate, oxalate, tannin) of extrudates from rice-sesame blends.

The specific objectives are to:

1. evaluate the proximate composition of the rice-sesame composites formed from fermented and sprouted rice and sesame seeds.
2. determine the nutritional properties of the extrudates produced from rice – sesame blends.
3. determine the anti-nutritional properties of the extrudates from rice – sesame blends.
4. evaluate the functional properties of the extrudates from rice – sesame blends.
5. conduct sensory evaluation of the extrudates from rice – sesame blends.

1.4 Justification for the Study

The production of cereals-legume based extruded products to supply additional protein and minerals to the daily diet of the vulnerable groups of the population has increased significantly over the years. Such products include nutritionally enhanced biscuits, breads, cakes, porridges and extruded snacks from wheat, corn, oats and rice. However, fewer rice-based extruded products are available in the market compared to those from other cereals such as corn and wheat (Danbaba *et al*., 2015). Nutritionally, rice is deficient in lysine, an essential amino acidthat can easily be improved by blending rice with food materials rich in lysine.

In addition, the industrial processing and utilization of legumes such as sesame have not been fully developed in Nigeria as its utilization is restricted to producing regions. For most part, the surplus crop is commercialized, bulked and exported with minimal processing limited to cleaning and drying (Makinde and Akinoso, 2013). Therefore, utilization of rice and sesame blends could be explored to produce affordable, nutrient dense, easily digestible products to overcome protein-energy-malnutrition among children. Although, studies about the nutritional characteristics of sesame seeds have been reported, there is little information on nutrient composition in value added products formulated with fermented, boiled, roasted and sprouted sesame seed flour (Akusu *et al*., 2020).

Hence, fermentation and sprouting could contribute in this direction, through the production of novel extruded foods containing cereals and legumes, with high nutritive value and acceptable by the consumers.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Nutrition

This is the sum total of the process by which living things receive and utilize the necessary materials for survival, growth and maintenance of worn out tissues. Food is defined as any solid or liquid which when ingested will enable the body to carry out any of its life function (Melkie, 2004). Most foods are made up of several simple substances, which we call nutrients. There are six nutrients each of which has specific function in the body. Those that supply energy are the carbohydrates and fats. Those responsible for growth and repair of tissues cells are proteins. Those, which regulate chemical process in the body, are the vitamins and minerals. Water is present in most foods and is an indispensable component of our bodies. It is the means of transportation for most nutrients and is needed for all cellular activities. The condition that results from an imbalance between dietary intake and requirements, which includes under nutrition, resulting from less food intake and hard physical work is known as malnutrition. Food containing all the nutrients in a sufficient amount and in proper ratio is referred to as diet.

2.1.1 Protein and amino acids in nutrition

Of the nutrients, proteins stay one of the most significant. Proteins are amongst the most important bio-molecules as they offer important roles in basically all life processes. Dietary proteins are the wellspring of essential amino acids and provide nitrogen for the synthesis of non-essential amino acids. Proteins in the body tissues are built using about 23 amino acids. Of these, 10 are fundamental amino acids which must be provided in the fish diet. Proteins or amino acids are important for upkeep, development, multiplication and substitution of exhausted tissues. Likewise, certain amino acids are readily converted to glucose to provide an essential energy source for some critical body organs and tissues such as brain and red blood cells (Poston *et al*., 2017). The functions of proteins cannot be over emphasized. They function as enzymes where they catalyze biological reactions. Proteins also serve as transporters in addition to aiding the storage of other substances as well as in provision of structural support along with protection by the immune system. The enhancement of movement of molecules within the body of living organisms is aided by proteins.

These significant macro-molecules are yield from a collection of 20 micro-molecules of amino acids. The tertiary structure and functions of these proteins relies largely on the amino acid sequence and the functional groups present. Depending on their functional groups and structure, amino acids are classified based on the nature of their side groups. These include basic, acidic, aliphatic, aromatic and hydrophilic amino acids (Jiri, 2014).

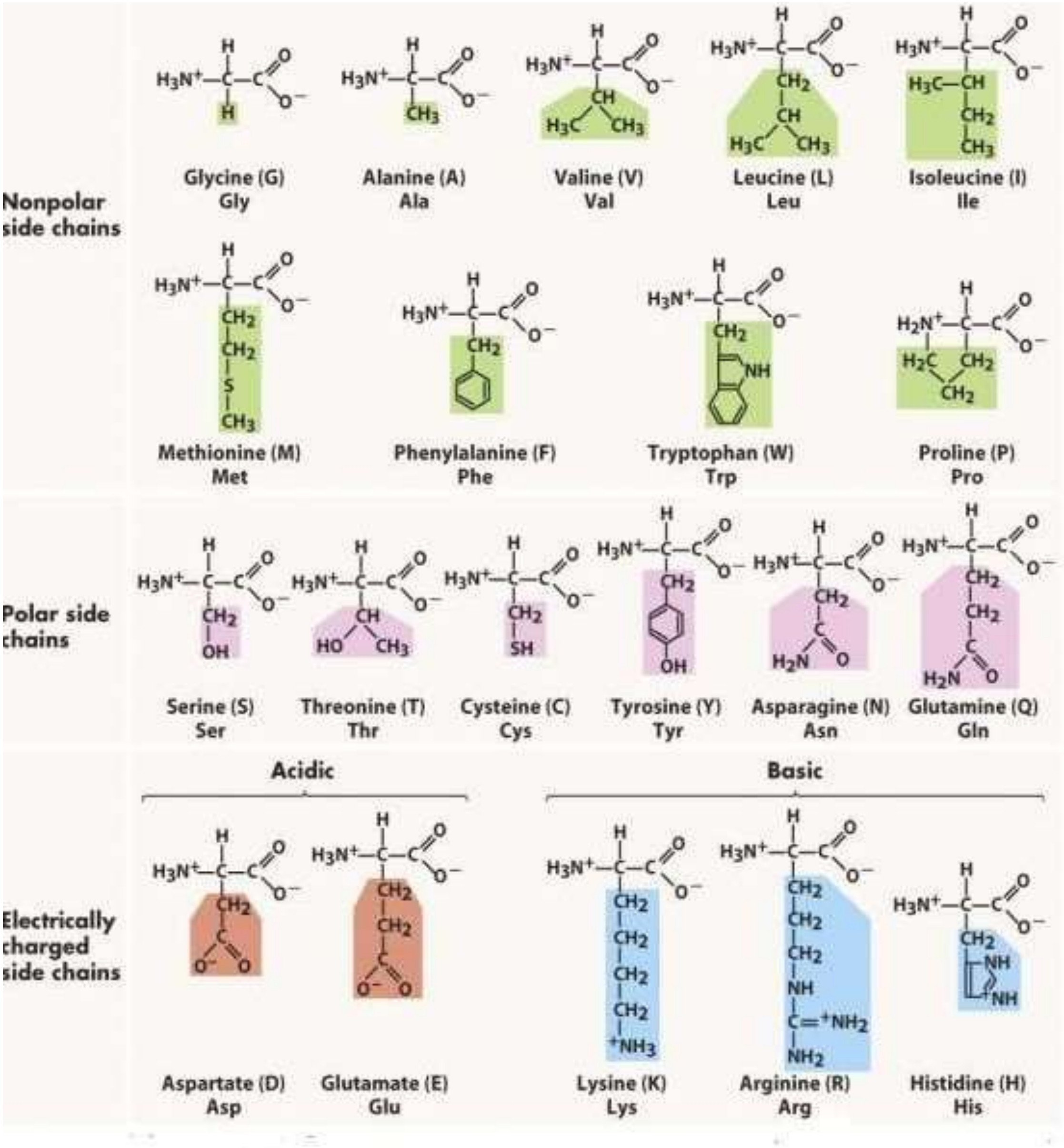

Figure 2.1: Structure of Amino Acids (Jeremy *et al.*, 2007)

Indispensable amino acids include Phenylalanine, Tyrosine, Isoleucine, Leucine, Lysine, Methionine, Cysteine, Valine, Threonine and Tryptophan. These are the limiting amino acids in animals. The animal systems can not to biosynthesize them and therefore, they must be supplied in diets. The remaining (non-essential) amino acids can be synthesized by animals. Dispensable amino acids like glutamic acid, aspartic acid, serine, alanine, glutamine, asparagine, tryptophan, glycine, are commonly available in the biological system of animal with a normal physiological states (Lopez and

Mohiuddin, 2020). However, at certain phases in life the synthesis of some amino acids becomes progressively limiting. Such stages include the period of intensive growth, stress or in some disease conditions. At such stages some amino acids called the semi essential amino acids must be necessarily provided in the diets. These include proline, cysteine, tyrosine and arginine. Thus, implies that adequate amounts for each essential amino acid are required in diets-and the more the amount of the essential amino acids present in a protein diet, the higher the protein diet quality (Ibironke *et al.,* 2012).

2.2 Protein-Energy Malnutrition

The World Health Organization (WHO, 2016) defines malnutrition as the cellular imbalance between the supply of nutrients and energy and the body's demand for them to ensure growth, maintenance of growth, and body specific functions. It is a condition that results from eating a diet in which one or more nutrients are either not sufficient or are too much such that the diet causes nutritional diseases. It may involve insufficient intake of calories, protein, carbohydrates, vitamins and minerals which most time leads to diseases. Nutritional diseases are diseases which outcome due to excess-nutrients or nutritional insufficiency than the normal requirements in animals (Idowu, 2002). A deficiency of essential amino acids may lead to poor utilization of dietary protein, and may result in growth retardation, poor live weight gain, and low feed efficiency. In severe cases, amino acid deficiency lowers resistance to diseases and impairs the effectiveness of the immune response mechanism. Deficiencies of specific amino acids may also elicit clinical signs. For example, experiments have shown that methionine deficiency is one cause of lens cataracts (Heinemann and Hildebrandt, 2021). To overcome these deficits in nutrients, intake of enriched or fortified food from either animals or plants must be encouraged. Combination of whole grain cereals, such as rice and sesame has the potentials to provide necessary nutrients for both young and old.

However, preparation process such as fermentation and sprouting could have some positive effects on the nutritional qualities of cereal foods.

2.3 Fermentation

The term fermentation is derived from the Latin word *fermentum*, which stands for boiling. It may be defined as any process for the formation of a product by the mass culturing of microorganisms (Stanbury, 2009). Fermented food preparation, as mentioned in literary texts, is more than 3,000 years old in India. The ethnic food fermentation process was modified continuously through the propagation of traditional knowledge and experiences from one generation to the next, particularly keeping in mind improved sensory qualities and safety. The idea of fermented food preparation also expanded with diverse locally available substrates including grains, vegetables, milk, fish, and meat products. The rural folk are found to prefer the fermented foods over the unfermented, because of their pleasant taste, texture, and color (Sekar, 2007).

Traditional fermented food preparation is one of the oldest biotechnological processes around the world in which microorganisms play a crucial role in improvement of sensory characteristics, bioenrichment, health promoting attributes, and preservation of foods. Fermentation helps to reduce nondigestible carbohydrates, enrich the pool of essential amino acids, vitamins and minerals, and increases the overall quality, digestibility, taste and aroma of the food (Pooja *et al.*, 2020). This extraordinary benefit of fermented food is helpful to maintain the healthy composition of intestinal microbiota essential for protection from various diseases and to maintain physiological homeostasis and the gut-brain relationship of the host. From this point of view, fermented food is designated as naturally fortified functional food. The term functional food was first introduced in Japan in the mid-1970s. It refers to processed foods containing physiologically active ingredients that aid specific bodily functions beyond basic nutrition. A recently proposed working definition of functional food is: food that can be satisfactorily demonstrated to affect beneficiallyone or more target functions in the body, beyond additional nutritional effect, in a way relevant to an improved state of health and wellbeing and/or reduction of risk of disease (Blandino *et al*., 2003). Global interest in cereal-based fermented products is increasing due to low fat/cholesterol, high minerals, dietary fibers, and phytochemical content. Beyond the basic nutrients, cereal-based fermented food confers several health promoting attributes, as it contains edible beneficial microbes, also called probiotics, fermentable sugars (of microbial and food origin, that is prebiotics), and digestive aids such as a group of microbe-derived hydrolytic enzymes. In addition, multi-strain or multispecies probiotics may provide greater beneficial effects than monostrain cultures. The synergistic actions of these exogenous microbiota create a sociable environment for commensals (native colonizing organisms), prevent the growth of otherwise enteropathogens, are beneficial for digestion and absorption, produce different metabolites including short chain fatty acids, especially butyrate, which have a positive effect on epithelial lining of the gastrointestinal tract, enhance mucosal cell differentiation, and this may also promote the immune barrier function of the epithelium, and on peristalsis, which improves transit (Granato *et al*., 2010). Cereal components are the natural growth media/carriers for probiotics and have a buffering capacity to protect the organisms in the harsh environment of the intestine. Considering these beneficial effects, the grain-based fermented foods have now become more popular than conventional dairy-based products, particularly in Japan and Europe (Saerela *et al*., 2002).

2.3.1 Fermentation procedure

Based on the source of microbes involved in the fermentation process, there are natural and inoculated fermentations. In addition, fermentation can be divided into solid-state fermentation (SSF) and liquid-state fermentation (LSF) according to the water content in the system. Edible seeds commonly need to be pretreated before fermentation, such as by soaking, cracking, milling, sieving and cooking. In many cases, these processing methods are combined together. Some basic principles involved in the fermentation process include.

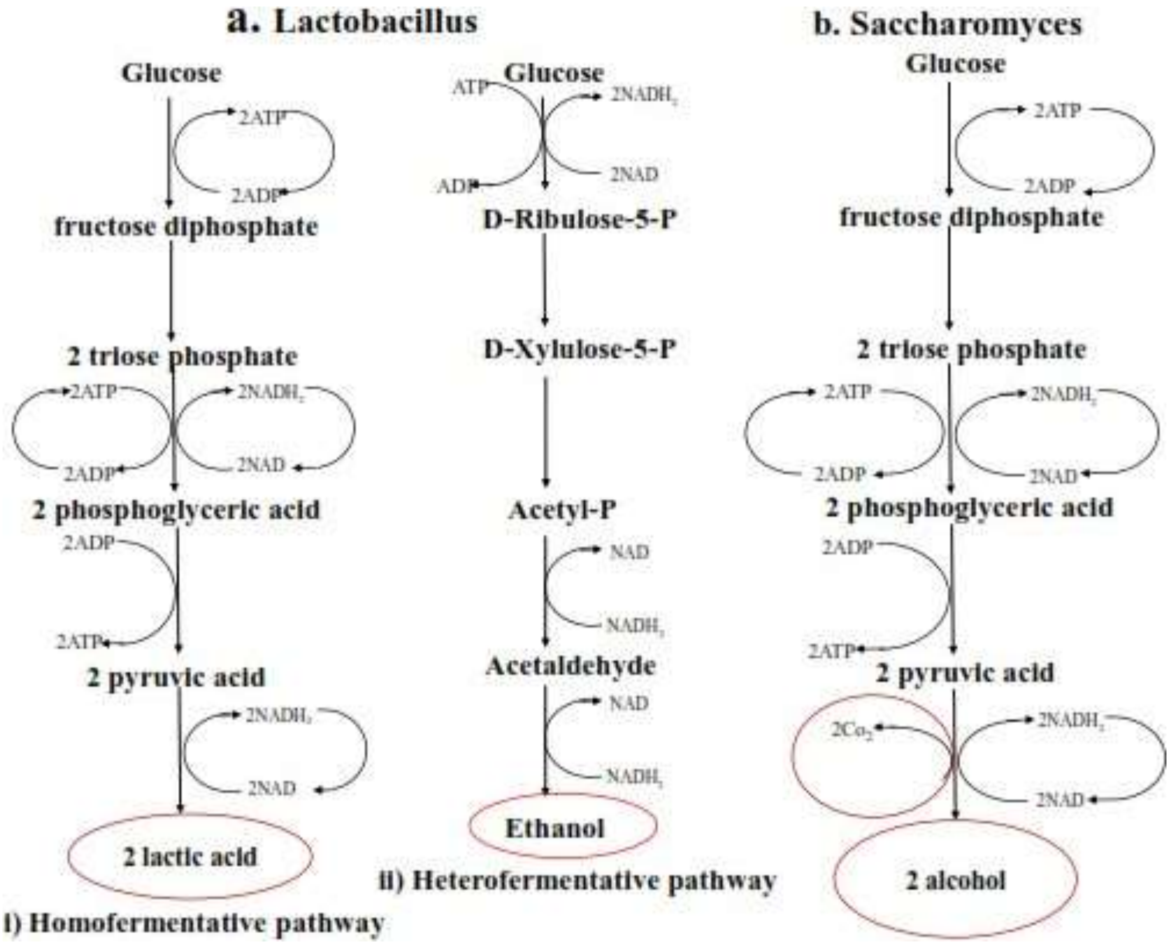

Figure 2.2: Sugar metabolism by Lactobacillus and Saccharomyces as representatives of L.A.B. and yeasts (Pfeiffer and Morley, 2014).

2.3.2 Pretreatment of edible seeds

Before the fermentation process, edible seeds should be pretreated. Fermentation commonly requires soaked, cracked, and cooked seeds or milled flour to start SSF or LSF. Of course, seeds cannot be cooked or autoclaved before natural fermentation since this will partly or completely kill microbes existing on seeds, leading to the failure of subsequent fermentation. On the other hand, it is better to reduce microbial populations existing on seeds before inoculated fermentation, since they would compete with and inhibit the growth of inoculated microbes during the fermentation process. For example, studies found that Lactic acid bacteria (LAB)-fermented soybeans and lentil also contain generally the same amount of aerobic mesophilic bacteria compared to LAB after 48 or 96 h of fermentation, suggesting the possible competitive relationship between aerobic mesophilic bacteria and LAB during fermentation. Therefore, edible seeds can be dried by hot air, such as at 40 to 60 °C for 24 h, or cooked/autoclaved, before the inoculated fermentation process. Overall, pretreatment of edible seeds can enhance the efficiency of fermentation.

2.3.3 Fermentation conditions

There are several factors, such as fermentation temperature, time, humidity, and other conditions, affecting the fermentation efficiency. Natural fermentation has been reported to control the temperature at 30, 37, or 42 °C (Elyas *et al*., 2002), which is probably associated with the main microbes carried by different seeds. In addition, the temperature is commonly controlled at 37 °C for LAB fermentation, while fermentation using bacillus subtilis and fungi has mostly been employed at 30 °C (Fernandez-Orozco *et al*., 2007), probably due to the optimum growth at this temperature. For fermentation time, several hours to several days has been reported, while 48 and/or 96 h is most commonly used for edible seed fermentation. In addition, it is better to control the fermentation humidity at 90 % to 95 % if possible, which can provide a relatively moist air condition for the growth of microbes. SSF of edible seeds and bean milk fermentation are generally performed quiescently, while LSF of edible seeds is commonly carried out by continuous shaking/stirring, with a speed of 200 to 450 rpm (Duenas *et al*., 2005), which can accelerate the growth of microbes, increase the interaction between microbes and substrates, and enhance the efficiency of fermentation. Similarly, adding sugars (1 % to 2 %) to the fermentation system, such as glucose or sucrose, can provide an extra energy source to accelerate the growth of microbes. Moreover, fermentation can be performed under aerobic or anaerobic conditions, depending on the species of microbes involved. For example, it is better to perform LAB fermentation in an anaerobic or microaerophilic environment. Overall, the fermentation condition is critical for the efficiency of fermentation and needs to be optimized for fermenting different products.

2.3.4 Treatment after fermentation

After fermentation, fermented edible seeds are commonly sterilized prior to further application. Autoclaving at 121 °C for 15 minutes coupled with subsequent freeze-drying is a common treatment (Gan *et al.*, 2016). In addition, hot air drying, such as at 70 °C for 3 to 4 h has also been used to treat fermented seeds. On the other hand, direct freeze-drying of fermented samples has also been reported, which may be better to retain nutritional and bioactive components. It should be mentioned that the step of sterilization after fermentation maybe associated with potential food safety problems.

2.3.5. Influences of fermentation on bioactive components

2.3.5.1 Vitamins

Vitamins are important essential nutrients. Based on solubility, they can be divided into water-soluble and fat-soluble groups. The former mainly includes the vitamin B groups and vitamin C, and the latter mainly contains vitamin A, vitamin D and E groups, and vitamin K. Edible seeds such as edible beans and cereal grains, are good natural sources of some vitamins, mainly the vitamin B and E groups (Fardet, 2010), while fermentation has distinct influences on different vitamins in edible seeds and their products. The vitamin B group mainly include thiamin (vitamin B_1), riboflavin (vitamin

B_2), niacin (vitamin B_3), pantothenic acid (vitamin B_5), pyridoxine (vitamin B_6), biotin (vitamin B_7), folicacid (vitamin B_9), and cobalamin (vitamin B_{12}). Fermentation has been reported to exhibit varying effects on vitamin B members in edible seeds and their products. Especially, fermentation by fungi and LAB, such as *Grifola frondosa*, *Rhizopus oligosporus* and *Lactobacillus acidophilus*, has been found to increase thiamin, riboflavin, niacin orpyridoxine in soymilk and buckwheat groats (Zhao and Shah, 2014).

This suggests that some microbes may possess the capacity of producing B group vitamins, consistent with previous studies that some LAB and fungi can synthesize B vitamins. The biosynthetic pathways of the main vitamin B group members in microbes have been reported (Magnusdottir *et al.* ,2015), Vitamin C (also known as ascorbic acid) generally occurs at low levels in edible seeds, and fermentation can cause opposite effects on its content in different edible seeds and their products. Fermentation was found to reduce ascorbic acid in lupin seeds and soymilk, while increasing it in red bean and buckwheat groats. This discrepancy maybe associated with the degradation or biosynthesis of ascorbic acid by microbes in the fermentation process, since different microbes possess the capacity of decomposing or synthesizing it (Bremus *et al.*, 2006). Several LAB have been reported to degrade ascorbic acid into simple organic acids, such as acetic and lactic acids, while yeasts, such as *Saccharomyces cerevisiae*, have been demonstrated to biosynthesize L-ascorbic acid from L-galactose (Hancock *et al.*, 2000). In addition, metabolically engineered yeasts have been reported to synthesize L-ascorbic acid from D-glucose, using the synthetic pathway found in plants. On the other hand, although *Rhizopus oligosporus*-fermented buckwheat groats and *Bacillus subtilis* combined with *Lactobacillus bulgaricus*-fermented red bean have been reported to increase ascorbic acid compared to unfermented samples (Jhan *et al.*, 2015; Malgorzata *et al.*, 2015), whether these microbes can biosynthesize ascorbic acid needs further investigation.

E vitamins include tocopherols and tocotrienols, each with α, β, γ, and δ homolog. In most edible seeds, γ-tocopherol is the most abundant vitamin E, with much higher content than other tocopherols. Fermentation has been reported to differently influence the contents of tocopherols in edible seeds andtheir products, probably associated with microbes involved in the fermentation process. Fungi, such as *Aspergillus oryzae*, *Rhizopus oryzae*, and *Rhizopus oligosporus*, have been found to increase tocopherols in soybean and buckwheat groats (Fernandez-Orozco *et al*., 2007). *Bacillus subtilis* was found to significantly increase γ and δ tocopherols in soybeans, while LAB exhibited different influences on E vitamins (Zhao and Shah 2014). These results suggest that some microbes may biosynthesize tocopherols during the fermentation process and a possible biosynthetic pathway of γ-tocopherol was proposed based on a previous study (Rippert *et al.,* 2004). On the other hand, the influence of fermentation on tocotrienols has scarcely been investigated.

In general, fermentation has distinct influences on various vitamins in edible seeds and their products, which can be associated with microbe-mediated biosynthesis or degradation of vitamins. In light of the health benefits of vitamins, it is promising to employ vitamin-producing microbes or genetically engineered microbes with enhanced vitamin-producing capacity to develop fermented edible seeds and their products rich in vitamins (Lee and Pan, 2012).

2.3.5.2 g-Aminobutyric acid (GABA)

GABA, a nonprotein amino acid, is an important inhibitory neurotransmitter in the mammalian nervous system, and it plays a critical role in the regulation of blood pressure and many other physiological functions (Diana *et al.*, 2014). It can be produced in plants, microorganisms, and mammals, and it is widely distributed in various foods. Considering the health benefits of GABA, the need for GABA-rich foods is increasing, and various food processing methods, such as fermentation, have been employed to enhance its content (Dhakal *et al.*, 2012). Edible seeds are natural sources of GABA, and many studies reported that fermentation can further increase GABA content in many edible seeds and their products, such as chickpea, faba bean, kidney bean, lentil, soybean, amaranth, buckwheat, millet, oat, quinoa, rice, rye, spelt, wheat, and/or their products. A variety of microorganisms has been employed in the fermentation process to enhance GABA content, and LAB are the most commonly used. In addition, fungi and molds, such as *Aspergillus oryzae, Rhizopus oryzae, and Monascus purpureus,* are also used to enhance GABA content in edible seeds (Lee and Pan, 2012).

Compared to sprouting where GABA is mainly synthesized by the seed itself, fermentation can accumulate GABA with the help of microorganisms. GABA is synthesized via glutamate decarboxylase (GAD)-mediated decarboxylation of glutamic acid, and GAD plays a central role in the synthesis of GABA (Battaglioli *et al.*, 2003). During the fermentation process, microbes can hydrolyze proteins and release free amino acids, such as glutamic acid, which can be used as the substrate for the synthesis of GABA by GABA-producing microbes. Many microbes have been reported to produce GAD to synthesize GABA, such as LAB and fungi (Wu and Shah, 2017). In addition, it is also possible that GABA can be synthesized by the endogenous GAD of seeds, since its concentration has been reported to be increased in control samples without inoculated microbes. Besides, many factors, such as fermentation temperature, pH, and fermentation time, as well as different media additives, can influence the production of GABA. Overall, fermentation is a valuablebioprocessing strategy for producing GABA-rich products (Dhakal *et al*., 2012).

2.3.5.3 Natural phenolics

Natural phenolics can be classified into different subgroups based on their chemical structures, such as phenolic acids, flavonoids, and proanthocyanidins. In plants, natural phenolics may exist in soluble and bound forms. Soluble phenolics include free and conjugated forms, with the latter conjugated with organic acids or sugar groups, and are synthesized through a multi-enzyme complex localized on the cytoplasmic surface of the endoplasmic reticulum, and are subsequently transported to intracellular vacuoles and stored there, while bound phenolics are formed by the secretion of vacuolar soluble phenolics to the cell wall, where they can covalently bind with cell wall macromolecules, such as polysaccharides and proteins, as a component of plant cell walls (Agati *et al.* ,2012). Edible seeds, such as edible beans and cereal grains, have been reported to contain a variety of natural phenolics. Phenolics mainly exist in the pigmented seed coats, primarily as flavonoids and proanthocyanidins, and many edible seeds contain a substantial level of bound phenolics, generally with much higher content than in common fruits and vegetables (Gan *et al.,* 2016).

Recent studies indicate that fermentation can change phenoliccomposition and distribution of edible seeds and their products. Most studies report that fermentation increases the total phenolics content (TPC) of soluble phenolics, while having different effects on specific phenolic compounds.

2.3.5.4 Bioactive peptides

Edible seeds, such as edible beans and cereal grains, are rich in proteins, which can be hydrolyzed into small-molecule peptides during the fermentation process. Recent studies have found that some fermented edible seeds and their products can produce bioactive peptides, mainly antioxidant and angiotensin converting-I-enzyme (ACE) inhibitory peptides, which are discussed below.

Antioxidant peptides exhibit various antioxidant activities, such as reducing free radical-scavenging, inhibition of lipid peroxidation, and metal ion chelation properties, which are mainly associated with the intrinsic characteristics of peptides, such as their amino acid composition, structure, and hydrophobicity. The main antioxidant peptides derived from fermented edible seeds and their products, and some intrinsic features and antioxidant mechanisms of antioxidant peptides have been proposed based on previous studies.

1. They, in general, contain 2 to 20 amino acids, all with molecular weight lower than 6.0 kDa.
2. The existence of amino acids, such as alanine, cysteine, histidine, lysine, leucine, methionine, proline, valine, tryptophan and tyrosine, may contribute to the antioxidant activity of peptides (Sarmadi and Ismail 2010).
3. Amino acid residues, including aspartic acid, glutamic acid, histidine, arginine, tryptophan and tyrosine can be associated with the chelating activity of antioxidant peptides.
4. Hydrophobic aminoacids can enhance the solubility of peptides in the oil environment, therefore facilitating the interaction with lipophilic radical species and polyunsaturated fatty acids (PUFAs).

5. The sulfur group (SH group) of cysteine and of methionine exhibits antioxidant activity, since it is able to neutralize reactive free radical species to form stable oxidation products, cystine and methione sulfoxide, respectively.
6. Histidine-containing peptides exhibit strong radical-scavenging activity due to decomposition of the imidazole ring (He *et al.*, 2012).
7. Histidine mainly acts as a chelator of metal ions at the amino terminus of peptides, while acting as an effective scavenger against various radicals at the carboxyl terminus.
8. Peptides with aromatic amino acid residues, including phenylalanine, tyrosine and tryptophan, are good donors of hydrogen and can efficiently scavenge free radicals due to their conjugated double bond structure of a benzene ring. These characteristics of antioxidant peptides can be helpful for predicting other potential antioxidant peptides, and they can also provide a reference for the chemical synthesis of antioxidant peptides. In addition, fermentation can also produce peptides with ACE-inhibitory activity in edible seeds and their products (Rizzello *et al.*, 2008). Overall, fermented edible seeds and their products contain variousbioactive components, especially vitamins, GABA, natural phenolics, and bioactive peptides, and these bioactive components endow the fermented products with versatile bioactivities. In the future, bioactive components in other fermented edible seeds and their products can be explored to provide a basis for the development of fermented functional foods.

2.3.6 Bioactivities of fermented edible seeds and their products

A large number of studies demonstrate that fermented edible seeds and their products exhibit manifold bioactivities such as antioxidant, anti-hypertensive, and anti-cancer effects.

2.3.6.1 Antioxidant effect

The influence of fermentation on the antioxidant effect of edible seeds and their products has been extensively investigated invitro. Various antioxidant effects, such as free radical-scavenging, and reducing and metal-chelating effects, have been reported to be increased in the hydrophilic extracts of most fermented edible seeds and their products compared to unfermented samples. The increase of antioxidant effect is mainly due to the increased antioxidant levels in fermented samples, mainly antioxidant phenolics and peptides. However, the influence of fermentation on antioxidant effect in the lipophilic and bound extracts of edible seeds and their products has been scarcely investigated. Unlike the hydrophilic extract commonly prepared by polar solutions, such as ethanol and methanol water solutions, the lipophilic extract needs to be prepared by nonpolar solutions, such as n-hexane or tetrahydrofuran, and the bound extract needs to be hydrolyzed by acidic, alkaline, or enzymatic solutions (Gan *et al*., 2016).

2.3.6.2 Anti-hypertensive effect

Hypertension is one of the most important risk factors for cardiovascular diseases. Blood pressure is tightly controlled by the renin–angiotensin–aldosterone system in humans. As a central component of the system, ACE functions to convert angiotensin into angiotensin II, which is able to increase blood pressure by directly causing blood vessels to constrict. As a result, ACE plays a central role in the control of blood pressure, and natural or synthetic compounds with ACE-inhibitoryactivity have been reported to lower blood pressure in experimental animals and humans (Fang *et al*., 2008). Recent studies demonstrate that some fermented edible seeds and their products exhibit anti-hypertensive effects in vitro and in vivo. However, the antihypertensive effect of fermented grains has been less investigated. Their bioactive components, such as GABA, ACE-inhibitory peptides, vitamins, and various antioxidant phenolics, can be responsible for the anti-hypertensive effect (Lee and Pan 2012). GABA and ACE-inhibitory peptides are found in many fermented edible seeds and their products, which have been reported to be predominantly responsible for their anti-hypertensive effect. As a result, fermented edible seeds and their products can be an important dietary component consumed by people to prevent hypertension. In addition, in light of the importance of cereal grains as staple foods for humans, more studies are needed to investigate the potential anti-hypertensive effect of fermented cereal grains in the future.

2.3.6.3 Other bioactivities

Fermented edible seeds and their products have also been reported to possess many other bioactivities, such as anti-depressant, anti-diabetic, anti-fungal, anti-inflammatory, antiobesity, anti-stress and fatigue, anti-trypanosomal, cardiovascular protective, gastrointestinal protective, hepatoprotective, neuroprotective, reproductive protective, skin protective, DNA-protective, heavy metal protective, laxative, immunomodulatory, gut microbiota regulatory, and sleep regulatory effects. Likewise, bioactive components, including GABA, phenolics, bioactive peptides, naturally occurring statins, nucleobases, amino acids, tocopherols, polyunsaturated fatty acids, monacolin K, dimerumic acid, and arabinoxylan, have been proposed to be associated with the bioactivities.

In general, fermented edible seeds and their products possess versatile bioactivities, implying that they should have comprehensive health benefits. Therefore, it is recommended to consumefermented edible seeds and their products as a part of the diet to prevent chronic diseases such as cancer and cardiovascular diseases.

2.3.7 Nutritional aspects of cereal-based fermented foods

2.3.7.1 Impact on food safety and shelf-life extension

Fermenting microorganisms employed to generate new products with improved sensorial and nutritional qualities often produce various metabolites that inhibit the growth of spoilage and/orpathogenic bacteria. These metabolites include organic acids such as lactic acid, propionic acid, acetic acid, etc. that decrease the initial pH value, creating an acidic environment in the food matrix and therefore, extends the shelf-life of the fermented product (Nyanzi and Jooste, 2012). Furthermore, ethanol and hydrogen peroxide, which are strong inhibitory factors for microbial growth, as well as other secondary metabolites that can act as antimicrobial compounds, are produced by some LAB and yeast species.

These metabolites can be effective in controlling fungal growth and mycotoxins production in grain matrices; the latter is of great importance for cereal derived products, raising public health concern since exposure to mycotoxins may cause adverse health effects to humans (Adebiyi *et al*., 2019). Lactobacillus and Pediococcus strains, possessing antimicrobial activities, were tested regarding their efficiency to reduce mycotoxin production from Fusarium as well as to restrain the growth of other mycotoxigenic fungi during malting of wheat grains (used for beverages and bakery products). LAB reduced the fusarium toxins (deoxynivalenol-vomitoxin-, T-2, HT-2, and zearalenone) by up to 75 % depending on the strain. Antifungal activity was also observed from LAB metabolites (especially from acetic acid and secondarily from lactic acid) (Juodeikiene *et al*., 2018). Antimicrobial peptides, bacteriocins, are produced by LAB and are partially related to the extended shelf-life of fermented products.

2.3.7.2 Enhancement in nutritive value and compositional changes of fermented cereal products

2.3.7.2.1 Protein and carbohydrate digestibility

Protein digestibility depends on the protein structure and the presence of antinutrient factors (protease inhibitors, phytases) that bind with them as well as other parameters such as pH, temperature and ionic strength, all of which are directly related to proteolytic activities. Fermentation may affect these factors and parameters and thereby contribute to a more effective digestibility of plant proteins (Joye, 2019). Proteins need to be broken down to amino acids or even small peptides to enter the human blood stream after their absorption by the enterocytes of the small intestine, otherwise they reach the large intestine where they are fermented by the gut microorganisms, giving rise to the formation of amines and short-chain fatty acids.

These fermentation products elicit various biological reactions via different receptors and mechanisms, including signal transduction involving biogenic amines as neurotransmitters and modulation of inflammatory responses (Fan *et al.*, 2015). Upon fermentation, microbial proteases are released and degrade to a certain extent the proteins included in a composite food matrix like cereal grains. Furthermore, the improvement of protein digestibility is accomplished through the reduction of antinutrient factors. Subsequently, the inactivation of digestive enzymes' inhibitors (trypsin and chymotrypsin inhibitors) in fermented cereal products is being discussed. The effect of fermentation on trypsin and chymotrypsin inhibitors was evaluated in bread samples made from various cereals (wheat, whole wheat, rye mix, and mixed flours). In the case of rye mix bread, the final trypsin inhibitors were almost half compared to the concentrations measured in the respective raw flour (Fan *et al.*, 2015).

Carbohydrates digestibility is of great importance as well since it is related to many human health issues. Starch digestion is directly linked to the glycemic index (GI) of foods and concerns diabetic and health conscious consumers who prefer products that do not rapidly raise glucose levels in blood and therefore avoid risks of insulin resistance and type 2 diabetes. Sourdough fermentation alters the cereal matrix through the production of lactic acid, which aids interactions between starch and gluten. These interactions result in reduced starch availability and a lower GI for the product (Melini *et al*., 2019). The most effcient lactobacilli (Lactobacillus amylovorus) regarding starch fermentation ability belong to the genera Lactobacillus, Lactococcus, and Streptococcus (Chaves-López *et al*., 2020).

Patients suffering from irritable bowel syndrome (IBS) are instructed to avoid cereals and follow a diet with low content in easily fermentative sugars. These sugars include Fermentable Oligo-Di-and Mono-saccharides and Polyols (FODMAP). Fructans, galactans, lactose, fructose, sorbitol, and mannitol are not absorbed in the small intestine and are rapidly fermented by gut bacteria when they reach the large intestine, thus inducing abdominal symptoms (diarrhea or constipation, swollen belly, meteorism, abdominal pain, etc.). During fermentation, LAB, yeasts, and fungi totally or partially degrade FODMAPs. Regardless of the extent of degradation, the decrease of FODMAPs is beneficial for people with IBS symptoms, since their adverse effects depend on FODMAPs' intake dose (Zanini and Arendt, 2018).

2.3.7.2.2 Dietary fiber modification

According to the American Association of Cereal Chemists (AACC), dietary fibers (DFs) are plant carbohydrates that are resistant to hydrolysis by human enzymes but can be fermented by microorganisms in the large intestine. They are classified in soluble and insoluble DFs and their ratio in food products plays a significant role in both health implications and physical–technological properties (Yegin *et al*., 2020).

Cereals contain various DFs, which chemically and compositionally differ depending on the type of cereal and grain tissue in which they are found. The main components of DFs of cereals are non-starch polysaccharides, that is arabinoxylans, O-glucans, cellulose, resistant starch, fructans, and lignin, which are a phenolic polymer and often exist in composite structures with other small molecular weight bioactives, for example, simple phenolics, minerals (Poutanen, 2020). During fermentation of cereals, the pH is decreased due to the production of organic acids (mainly lactic and acetic) and this may result in the activation of various enzymes, either endogenous of the grains or bacterial. The enzymatic activity is responsible for biopolymer degradation, leading to grain softening (cell wall degradation) and improvement of the sensory and physiological characteristics of the fermented product (Poutanen, 2020).

2.3.7.2.3 Vitamins

Vitamins play a crucial role in proper metabolic functions and therefore, their daily intake is essential since they can not be synthesized in adequate amounts (or at all) in the human body. These essential micronutrients are divided into two sub-categories according to their solubility: a) the water-soluble vitamin C and the group of B vitamins (thiamin-B$_1$, riboflavin-B$_2$, niacin-B$_3$, pantothenicacid-B$_5$, pyridoxine-B$_6$, biotin, folic acid, cobalamin-B$_{12}$), and b) the fat-soluble vitamins A, D, E, and K (Gan *et al.*, 2017). Although cereals contain specific vitamins, fermentation with LAB or yeast strains can increase their vitamin content. The ability of LAB to produce vitamins is strain-specific and these microorganisms could be used as starter or added cultures to fortify naturally fermented products for targeted nutritional and quality improvement. Such a fortification is of great importance for specific population groups that follow special types of dietary regimes, either by choice (for example, vegans) or due to cultural habits, religious beliefs, and lack of other available food sources (developing countries).

It is worth mentioning that in some African countries, porridges made of cereals can complement breastfeeding (Gabaza *et al*., 2018). Biofortification in vitamins of fermented cereal products has been attempted by a few researchers. For example, the incorporation of *Lactococcus lactis* N8 and *Saccharomyces boulardii* SAA655 in idli batter (an Indian steamed cake made from rice and legumes) increased the riboflavin and folate content by 40 – 90 %. Furthermore, there are limited reports on the effect of bacterial and yeast/fungal fermentation of cereals on vitamin E concentration (Pozzo *et al*., 2015).

2.3.7.2.4 Phenolic components

Phenolic components, which are secondary plant metabolites, are also found in notable amounts in cereals. The metabolic pathways (shikimate, phenylpropanoid) for their biosynthesis involve many biomolecules like acetyl CoA, malonyl CoA, pyruvate, acetate, and some amino acids (phenylalanine and tyrosine) (Adebo *et al*., 2019). Their beneficial properties in human health (anti-diabetic, anti-cancer, anti-inflammatory, anti-microbial, anti-oxidant, as well as neuro-, cardio-, and hepato-protective function) are attributed to their ideal chemical structure, which promotes electron transfer or hydrogen donation from the hydroxyl groups of their aromatic ring and thereby exhibit free radical scavengingactivities and metal-chelating potential. Phenolic components need to be in a soluble form to enter the human blood circulation system and bring about their antioxidant properties. Phenolics in cereals can be found as free and soluble, conjugated and soluble (bound with sugars and sterols), and non-soluble, which are usually linked to polymers like arabinoxylans and lignin. Increases ofcereals' phenolic content can be achieved by size reduction of the particles, sprouting, addition of hydrolytic enzymes, and fermentation. Fermentation is reported to also enhance the antioxidant activity of the phenolic fraction (Acosta-Estrada *et al*., 2014). The conditions during fermentation (temperature, final pH value and duration), microorganisms involved, as well as the type of cereal and the grain tissue employed play an important role in the outcome concerning the release of the bound phenolics.

2.3.7.3 Reduction of antinutrients and allergens

Besides valuable nutrient compounds, cereals contain a notable number of components that are considered anti-nutritional factors (ANFs). These components include phytic acid/phytate (myoinositol-1,2,3,4,5,6-hexakis dihydrogen phosphate), tannins, and polyphenols (Melini *et al.*, 2019). Phytate or phytic acid is a secondary metabolite, found mainly in the aleurone layer and pericarp (wheat, rice) or in the endosperm (maize) of cereals, serving as their phosphorus repository. The content of phytate in cereals varies between 0.18 g to almost 6.5 g per 100 g product (dry weight). The presence of phytic acid plays a crucial role in the nutritional value of the food in which it is found as it has the ability to hinder enzymatic activity (trypsin and beta-galactosidase) and form chelates with metal ions, that is, iron, magnesium, calcium, and zinc, thus reducing their bioavailability. Likewise, tannins and polyphenols have a strong negative effect on protein digestibility since their hydroxyl groups form complexes with the carbonyl group of proteins. As a result, proteins precipitate, proteases are inhibited, and thereby, amino acid deprivation is realized when a diet is based on cereal products rich on polyphenols, which is the case in most developing countries or for people following a vegan dietary pattern (Joye, 2019). Prolonged periods of these nutritional deficiencies may also lead to osteoporosis, iron deficiency anemia, and impairments of physical growth.

Among the technologies and strategies applied for ANFs reduction, fermentation is considered one of the most effective ones. Mineral binders were reduced by fermentation in togwa, sorghum porridges, and finger millet porridges (Gabaza *et al*., 2018). Phytic acid can be degraded by endogenous or microbial phytases during natural fermentation and increase the bioavailable amount of iron, calcium, and zinc. The value of pH encountered for fermented products is considered optimal for the enzymes to act against phytate (Gupta *et al*., 2015). Wet and solid-state fermentation of various cereals and their brans have been studied considering the physicochemical alteration of the products and the effect on dephytinization.

2.4 Sprouting

The term sprouted seeds involves different types of products obtained from seeds, depending on the part of the plant collected and consumed, in particular whether the seed is comprised or removed, and on the growing substrate and environmental conditions during sprouting. For each of these products several ambiguous commercial definitions occur (that is, microgreens, shoots, babygreens, cress, wheatgrass), widespread even in the scientific literature, and the same term could refer to different types of product. This frequently leads to misunderstanding, depriving specialists of the basic terminology on which it is necessary to point out, since the only legal definition in Western countries is given for sprouts and sprouted grains.

Sprouts are the product obtained from the sprouting of seeds and their development in water or another medium, harvested before the development of true leaves and which is intended to be eaten whole, including the seed. Sprouted grains are defined by the American Association of Cereal Chemists (AACC) with the endorsement of the United States Department of Agriculture (USDA) as follows: malted or sprouted grains containing all of the original bran, germ, and endosperm shall be considered whole grains as long as sprout growth does not exceed kernel length and nutrient values have not diminished. These grains should be labeled as malted or sprouted whole grain (AACC, 2008).

Basically, it is essential to clarify that:

1. shoots are intended to come from the sprouting of seeds in water, to produce a green shoot with very young leaves and/or cotyledons the final product does not include the seed teguments and the root
2. cress is the shoots grown in soilor hydroponic substrate, sold as the entire plants (BIOHAZ, 2011; ESSA, 2016).
3. microgreens are the most common and equivocal market term, it may be referred to as seedlings at fully expanded cotyledon stage, or at the first true leaf stage, sold with stem, cotyledons, and first true leaves
4. wheatgrass is the youngest stage of wheat plant which grows from the wheat grain and takes 6 – 10 days to germinate (Pant *et al*., 2013).

2.4.1 Use of sprouted seeds in human nutrition

Sprouting of seeds has been known for a very long time, mainly in the Eastern countries where seedlings are traditionally consumed as an important component of culinary history. Starting from the 1980s, the consumption of sprouted seeds raised popularity also in theWestern countries due to the consumer demand for dietetics and exotic healthy foods; in the latest years the interest around sprouted seeds has been focusing principally on low processing and additive-free. Given their peculiar characteristics such as unique color, rich flavor and appreciable content of bioactive substances, they could be used to enhance the sensorial properties of salads, or to garnish a wide variety of high-quality products (Treadwell *et al*., 2020). Moreover, sprouting is a simple and inexpensive process which can be done without sophisticated equipment, has a quick production cycle (two to three weeks at most), occupies very little space in greenhouse production and provides fairly high yields (Lorenz and D'Appolonia, 2018). Besides malting; which represents a special kind of Sprouting used for the production of alcoholic beverages, cereal seedlings might be consumed in the form of ready-to-eat sprouts or further processed, for example, dried or roasted (Hübner and Arendt, 2013). A possible trend is the supplementation of wheat bread in flour from sprouted cereals and pseudocereals. However, the high accumulations of enzymatic activity under uncontrolled sprouting conditions may adversely affect the physical properties of dough and the resulting baking performance, making the use of sprouted cereals for baking more challenging (Marti *et al*., 2018). The dehydrated sprouted cereals can be also used for making noodles, pasta, unleavened bread and porridge. Functional beverages which are obtained by lactic acid fermentation of mixture based on sprouted grains and flour, represent a possible future perspective (Sharma *et al*., 2014). Indeed, cereals contain water-soluble fiber, oligosaccharides and resistant starch, and thus have been suggested to fulfill the probiotic formulations. Further perspectives could be given by the use of cereal sprouts as supplements in animal feeding, as it has been proposed for non-grain species (Dal Bosco, 2015).

2.4.2 Changes in chemical composition during sprouting

By definition, sprouting incorporates those events that begin with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryo axis, usually the radicle, which extends to penetrate the structures that surround it (Bewley, 2007). The subsequent mobilization of the major storage reserves is associated with the growth of seedling. Therefore, physical and biochemical events underlie this process, that is, weakening of seed covers, turning on of metabolic activity, activation of gene transcription, relaxation of the embryonic cell walls, and reassembly and biogenesis of organelles (Logan *et al*., 2001).

Briefly, during a first phase (Phase I) there is a rapid imbibition of water by the dry seeds until all of the matrices and cell contents are fully hydrated. Then, a second phase (Phase II) involves a limited water uptake (plateau phase) but a strong metabolic reactivation. The increase in water uptake associated with Phase III is associated with cell elongation leading to completion of sprouting (Bewley, 2007). Upon imbibition, the quiescent dry seeds rapidly restore their metabolic activity, including remobilization, degradation and accumulation, which imply important biochemical, nutritional and sensorial changes in the edible products (Dziki *et al*., 2015). The out coming primary and secondary metabolites exert differential biological health effects when consumed compared with non-sprouted seeds.

2.4.3 Carbohydrates

2.4.3.1 Non-structural carbohydrates

Since one of the most studied processes on seedling development is the mobilization of complex polymers, such as starch, the substantial alterations in grain carbohydrates have been extensively studied in most of the sprouted grains (Aoki *et al*., 2006). In germinating grains, amylases catalyze the hydrolysis of starch, stored as amylose and amylopectin, to simple sugars, that is, the reducing sugars glucose and maltose and, to a lesser extent, the non-reducing sugar sucrose, resulting in a higher digestibility (Chung *et al*., 2012).

However, trends in sugar profile of sprouted grains mainly depend on species: rice, sorghum, and millet seem to accumulate more maltose than glucose, while wheat greatly accumulates glucose instead of maltose (Chiba *et al*., 2012). Differential effects have also been reported in response to Sprouting time, with sucrose as the dominant source of carbohydrate during early wheat sprouting phase and glucose and maltose during the later stages (3 days post-imbibition) also, in rice, sucrose predominated shortly after imbibition (Aoki *et al*., 2006). However, sprouted rice seedlings show high sucrose levels for a long post-imbibition period, glucose content increases more rapidly, and maltose do not appear at a significant level until much later (7 days post-imbibition). Sprouted grain tissue also plays a significant role: higher glucose content is located in the endosperm of wheat and rice, compared with sucrose in the scutellum (Aoki *et al*., 2006).

2.4.3.2 Structural carbohydrates

Dietary fibers represent an important component of the whole grain. Cellulose, hemicelluloses and lignans are water insoluble fibers, while *b*-glucans and arabinoxylans (AXs) are grouped as water-soluble dietary fiber although they are either water extractable or water unextractable. Barley (5 – 11%) and oats (3 – 7%) are particularly rich in *b*-glucans, as well as sorghum and millet, while other cereals species contain only lower amounts (Lemmens *et al*., 2018).

The effect of sprouting on dietary fiber content of sprouted grains is often inconsistent and strictly depends on fiber fraction, sprouting time and genotypes. In rice, an increase in total dietary fiber after malting is observed and could be explained with the formation of new primary cell walls (Lee *et al*., 2007). Besides the increase in total fiber content, an upward trend has also been recorded for both soluble and insoluble fiber fractions of sprouted brown rice varieties, while the ratio of soluble vs. insoluble ones varied in response to genotype and processing conditions (Hübner and Arendt, 2013).

The extent of changes in AXs content during sprouting has garnered much less attention than *b*-glucan. AXs are non-starch polysaccharides, found as cell wall constituents and esterified with ferulic and p-coumaric acids. In general, the total AXs content is not significantly affected by sprouting. However, significant lower content in AXs has been associated with the sprouting of oats and rye; during malting of barley. On the other hand, the amount of water extractable AXs can be increased during sprouting by the release of soluble AXs previously covalently bound to other cell walls, resulting in altered proportions of total to water extractable AXs.

2.4.3.3 Proteins

Whole grains major storage proteins of most cereals are classified according to their solubility properties into albumins (water-soluble), globulins (salt-soluble), glutelins (alkali-soluble), and prolamins (alcohol-soluble). During grain sprouting, the storage proteins are hydrolyzed into peptides and amino acids by proteolytic enzymes after 2–3 days from imbibition, thereby increasing nutrient bioavailability (Taylor *et al.*, 2015). It is well known that prolamins content decreases as time of sprouting increases, as observed in triticale, barley, rye, oats and wheat. On the other hand, several reports show an increase in crude proteins in barley, waxy wheat, brown rice and oats, as a result of the proteins and amino acids stored in the cereal being decomposed by water absorption, changes into transportable amides, and supplied to the growing parts of the seedlings (Singkhornart and Ryu, 2011). However, the protein content depends on the balance between protein degradation and protein biosynthesis during sprouting. Besides an increase in amino acids contents, significant alterations in free amino acid composition have been observed. In particular, sprouted whole grains contain higher quantities of essential amino acids, which take part in protein production in human body. Grain type and sprouting time have the greatest influence on the amino acid composition (Rusydi *et al*., 2011). Rice and buckwheat malts produce higher amounts of amino acids after 5 days of sprouting. This is characterized by very low amounts of asparagine, methionine and histidine. In oats, an increase in albumin content (rich in essential amino acids lysine and tryptophane) and a subsequent decrease in globuline and prolamine contents (poor in lysine) have been observed during sprouting (Kaukovirta *et al*., 2004).

2.4.3.4 Lipids

Even in cereals, where starch is the main carbon store in the endosperm, lipids are abundant in the living tissues of whole grains (that is, embryo, scutellum and aleurone) as oil (triacylglycerols, TAG). The mobilization of TAG from oil bodies requires a coordinated metabolic activity, which is started on with sprouting, leading to the net conversion of oil to sugars (Graham, 2008). The lipases firstly release the esterified fatty acids (FAs) from TAG. Free fatty acids (FFAs) can then be degraded through the *b*-oxidation and glyoxylate cycles and subsequently converted into sugars (Graham, 2008).

2.4.3.5 Phytate and minerals

Phytate is highly concentrated in several food items derived from plants; it represents the major storage form of phosphorus in mature grains and legumes. However, in human beings the insufficient endogenous intestinal phytase limits phosphorus utilization; phytate also negatively impacts the bioavailability of mineral ions such as Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, Mn^{2+} and Cu^{2+} since it is characterized by a strong chelation affinity with cations and is therefore considered an antinutritional factor (Kumar *et al*., 2010). Phytases are a subfamily of the high-molecular-weight histidine acid phosphatases, involved in the hydrolysis of phytate to myo-inositol and ortho-phosphate, as well as inorganic phosphate. The phytase activity tends to increase during sprouting; in barley. However, the concentration of phytase in whole grains greatly varies among cereal species, with rye having the highest values and oats the lowest ones (Hübner and Arendt, 2013). As a consequence, Phytate content diminishes to a different extent during Sprouting. Further studies on phytate degradation during sprouting processes have been reported for brown rice, barley, pearl millet, corn, sorghum and wheat. As phytate content decreases, bioavailability of phosphorus and minerals increases (Azeke *et al*., 2011).

2.4.3.6 Antioxidants

Whole grains contain high concentrations of antioxidants, such as polyphenols, carotenoids, ascorbic acid and tocopherols, which balance oxidative damages of seedling cell components (Bailly, 2004). Phenolic acids in seeds are present in both free and bound fractions, with the latter as the most representative, linked to hydrolyzable tannins, lignins, cellulose and proteins, which are mainlystructural components of bran and aleurone (Engert *et al*., 2011). Generally, sprouting leads to a mild increase of the total polyphenols content, although different contribution from free and bound fractions is observed, depending on species and sprouting conditions. The free fraction increases and the bound fraction decreases as sprouting goes on (Benincasa *et al*., 2015). Indeed, ferulic and p-coumaric acids, which greatly contribute to the total bound fraction, are known to be involved in cell wall structure and development (Ralph *et al*., 2014) Conversely, in brown rice both free- and bound- fractions significantly increases over

Sprouting time; this is probably due to the hydrolysis of conjugated phenolic compounds and to contemporaneous de novo biosynthesis in the embryo axis. Tartary buckwheat is naturally rich in flavonoids such as rutin, quercetin and catechin; in 8-day sprouts, high rutin and catechin and lower quercetin concentrations were observed with respect to whole grains. Interestingly, oats are unique among cereals containing avenanthramides (AVAs) that is, low molecular weight soluble phenolic compounds which has been reported to increase by approximately 20 % during sprouting (Hübner and Arendt, 2013).

2.5 Factors Influencing Nutritional Quality of Sprouted Whole Grains

2.5.1 Genotype and seed source

The most significant role in the determination of nutritional value of sprouted grains is played by the genotype. In the last years, several studies focused on the characterization of grains from different ancient and modern cereals genotypes as well as pseudocereals species, principally in terms of bioactive compounds (Sompong *et al*., 2011).

As is known, the biochemical composition of whole grains is also conditioned by the environmental condition during crop growth, especially during grain development. Environment has been indicated as the main factor contributing to the total variation in some quality parameters in the case of winterand spring wheat varieties. Different effects of abiotic stresses during ripening (that is, droughtor high temperatures) have been observed, with increases in protein and carotenoid contents, and contrasting effects on starch quality (Singh *et al*., 2008). This is generally because dehydration stress shortens the grain filling period.

The nutritional value of wheat grains is also influenced by the exposure of the mother plant to biotic stress (that is, pathogens, weeds) and nutrient shortage. Many studies, focusing on the differences in nutritional values between organic and conventional cereals, have led to contradictory results (Mazzoncini *et al*., 2015). Although phenolic compounds accumulate more under biotic stress conditions, and consequently organic crops are often thought to contain more phenolic compounds, the effect of cultivation system on secondary metabolites content is often not significant, also in terms of single phenolic acids. The influence of different agronomic practices or environmental stresses on secondary metabolites in the field is naturally modified by the effects of other potential co-variables (Aloisi *et al*., 2016).

2.5.2 Sprouting conditions

Biochemical changes during sprouting occur depending on sprouting conditions as well as on the seed invigoration treatments applied to the grains in order to improve the sprouting and post-sprouting seedling growth. Seed priming is a pre-sowing treatment during which seeds are hydrated with a solution that allows them to imbibe and go through the first reversible stage of sprouting but does not allow radicle protrusion through the seed coat (Lutts *et al*., 2016). Common priming techniques include osmopriming (soaking seeds in osmotic solutions such as polyethylene glycol, PEG), halopriming (soaking seeds in salt solutions) and hydropriming (soaking seeds in water).

Recently, researches were addressed to identify the optimal combination of temperature and time during pre-sowing and sprouting treatments, to obtain higher quality sprouts, especially in terms of phytochemical content. These goals are achieved through the use of sophisticated statistica ltechniques including the response surface methodology approach. For example, in Ecuadorian brown rice cultivars, soaked grains (deionized water, 28 ^{o}C, 24 h) were introduced in a sprouting cabinet at 28 and 34 ^{o}C in darkness for 48 and 96 h (Cáceres *et al*., 2014). The multiple linear regressions predicted optimal sprouting conditions for accumulation of GABA and antioxidant activity after soaking followed by sprouting at 34 ^{o}C for 96 h, while the highest total phenolic content was obtained in the combination of 28 ^{o}C for 96 h, although differences between genotypes were recorded. In foxtail millet the highest total phenolic content, total flavonoid content and antioxidant activity were obtained with 15.84 h of soaking in tap water at room temperature and 40 h of sprouting at 25 ^{o}C (Sharma *et al*., 2015).

The optimum sprouting conditions for sorghum suitable for supplementary food formulations (that is, low tannin and high protein contents) were established to be steeping for 24 h at 31 ^{o}C plus sprouting for 4 - 5 days at 30 ^{o}C. The highest antioxidant concentrations in wheat sprouts were achieved following 7 days of sprouting at 16.5 ^{o}C while in purple corn sprouts it was possible to maximize the content of GABA, total phenolic compounds and antioxidant activity using a sprouting temperature of 26 ^{o}C for 63 h. The same conditions were guaranteed by kiwicha (*Amaranthus caudatus*) sprouts, which are richer in GABA and phenolic compounds, while the highest phenolic content in sprouted quinoa was obtained at 20 ^{o}C for 42 h (Paucar *et al*., 2018). The results reported here refer to specific experiments conducted in controlled environments, under specific laboratory conditions and sprouting times, so that the stage of cereal grass is not always reached. Accordingly, the optimization of seedling growth parameters needs to be deeply investigated. Not always pre-sowing treatments induce a greater accumulation of bioactive compounds. During the 24 h soaking period, an increase of total phenolic compounds was observed in brown rice. The pH of the soaking solution can affect the enzymatic activities in seeds which may enhance or reduce the phytochemical content in sprouted grains. In brown rice the optimal pH for higher GABA content ranges from 3.0 to 5.8, since a lower cytosolic pH stimulates GAD activity.

Sub-optimal conditions during sprouting process can lead to an accumulation of phytochemicals in seedlings due to the activation of secondary metabolism (Zhang *et al*., 2014). Rehydration involves high levels of oxidative stress, so that abiotic stress induced during seed sprouting can intensify the production of reactive oxygen species (ROS), which could damage the structures of DNA, protein, lipid, and other macromolecules in the seeds. Therefore, ROS scavenging is pivotal for seed sprouting under stress conditions and comprises non-enzymatic components, mainly linked with overproduction of antioxidants (for example, phenolics) an induced environmental stress during sprouting can be classified as abiotic elicitor (Liu *et al*., 2019).

It is important to highlight that the stressful conditions during sprouting, as well as the pre-sowing grain treatments, may reduce sprouting percentage and/or dry matter production. It follows that the commercial use of those manipulations of environmental conditions during sprouting should be appropriately set up for each species, to obtain sprouts characterized by higher nutritive and health promoting values, without or slightly affecting the production levels (Pardhi *et al*., 2019).

2.5.3 High and low temperatures

Few studies investigated on the effects of extreme temperatures applied during sprouting process on the nutritional quality of sprouted seeds; most of these researches focused on non-gramineous species like lentil, broccoli, alfalfa and radish (Oh and Rajashekar, 2009). In soft white winter and dark northern spring wheats, the GABA content increased during 48 h of sprouting and after sequential hydration with anaerobic and heat treatments; additionally, cold stress has been also reported to regulate the biosynthesis of anthocyanins in various plants, including 12-day old tartary buckwheat seedlings, grown for 4 days at 4 ^{o}C (Li *et al*., 2015). Furthermore, anthocyanin-rich sprouts display brighter colors, which help improve their nutritional and health profiles attracting more consumers (Pardhi *et al*., 2019).

2.5.4 Light modulation

Light is one of the main factors affecting plant growth and development. Photoreception systems respond to light intensity and quality as well as to light duration and intermittence, thus determining plant morphogenetic changes, functioning of the photosynthetic apparatus, and trend of metabolic pathways. Moreover, lighting conditions might evoke the photoxidative changes in plants, which lead to an altered action of the antioxidant defense system (Samuoliene *et al*., 2011). Despite the effects of the light irradiance level are well documented, data regarding the effect of light spectral quality in plantmetabolism are still limited (Pardhi *et al*., 2019).

2.5.5 Salt stress

Salinity causes one of the most important abiotic stresses in plants, especially during the early seedling growth, which is a very salt-sensitive phase. However, to date, little is known about the effect of salinity on the phytochemical accumulation in edible sprouts since the impact of salt stress has been principally evaluated in terms of sprouting rates and physiology (Falcinelli *et al*., 2017). Studies on grains are limited, so that an accurate analysis of the current literature over this topic needs to include evidences from some non-gramineous species.

2.5.6 Hypoxia stress

The alteration of gas composition during sprouting has been investigated in several species. In particular, a GABA accumulation in seedlings, in response to hypoxia stress during sprouting was observed in soybean, faba bean and brown rice (Ding *et al*., 2013). The stimulation of GABA synthesis is an adaptive response of plant tissues to stress-induced cytosolic acidosis, associated to the oxygen deficit stress (Aurisano *et al*., 2015). Indeed, hypoxia stress is often investigated in combination to acidic culture solutions as sprouting substrates (Guo *et al*., 2016).

2.5.7 Biofortification

Sprouted whole grains would be a promising vehicle for food biofortification programs. In brown rice, a recent research by Wei *et al*. (2013) has shown the possibility to increase Fe concentration in sprouted grains (24 h of sprouting) by soaking kernels in solutions of $FeSO_4$, just before sprouting process. Fe fortification increased Fe concentration of 1.1 – 15.6 times in brown rice sprouts, due to the penetration of Fe solutions across the aleurone layers via the dorsal vascularbundle present in the endosperm, as well as Fe solubility, which was nearly 4-fold higher than in non-fortified sprout (Wei *et al*., 2013). However, the relatively low permeability of some seed coats does not allow obtain fortification with Fe enriched solutions. This was the case for broccoli and radish soaked with Fe(III) - EDTA and Fe(III) - citrate solutions. Conversely, it was also observed significantly higher iron concentrations in 5-day old alfalfa sprouts obtained from Fe-soaked seeds. This increase was associated with a significant decrease in Ca, Mg, Na and or Mn concentrations, due to the leakage during imbibition, and with a significant induction of phenolic compounds concentration (Park *et al*., 2014).

Plant seeds are able to accumulate Se and to transform it from inorganic to organic form (that is, Se-containing proteins) during sprouting. In this way, Se-biofortification during sprouting could represent a valid strategy to improve Se concentration in seedlings (D'Amato *et al*., 2018). Fortification programs can also be applied during crop cycles, representing a suitable approach to ameliorate the concentration of macro- and micro-elements in whole grains, thus influencing their dynamics during the subsequent sprouting process (Pardhi *et al*., 2019).

2.5.8 Effects of sprouting on other cereals and pulses

Studies have shown the enhancement of nutritional quality of wheat and soybean seeds on sprouting. Chauhan and Chauhan (2007), observed that 4 days of sprouting of soybean caused a reduction in anti-nutrients and that the sensory attributes of soy beverages prepared from sprouted soybean decreased with increase in the time of sprouting. Guleria *et al*. (2009) studied percent sprouting, vigour index and lipid composition of sprouted seeds located at different node positions of soybean stem axis. Their study revealed significant differences in lipid composition as a function of nodal position of stem axis. The seeds present at the basal nodes showed higher sprouting percentage, while the vigour index was higher at apical position. Chauhan and Chauhan (2007) studied the effect of sprouting on anti-nutrients like phytic phosphorus, raffinose, stachyose, trypsin inhibitor activity and saponins in soybean. Sprouting for 2 days was observed to be optimum for producing soy beverage without any significant change in the sensory attributes and the beverage was devoid of oligosaccharides.

Murugkar and Jha (2009) observed sprouting ability as well as changes in nutritional and functional qualities on sprouting of four cultivars of soybean commonly grown in India viz. MAUS-47, DSb1, MACS 450 and JS 9305.They sprouted the cultivars at 25

°C with 90 % relative humidity for 48 and 72 h and observed that MAUS 47 and JS 9305 showed maximum potential in terms of their sprouting capacity and length of sprouts, and also maximum decrease in fat, trypsin inhibitor and phytic acid contents. The functional qualities of sprouted soybean varieties were also superior. Varieties MAUS 47 and JS 9305 were found to be most suitable for sprouting. Chopra *et al*. (2009) studied the varietal differences and effect of soaking and sprouting on insoluble (IDF), soluble (SDF) and total dietary fiber (TDF) of Bengal gram, cow pea, dry pea, field bean and green gram. Samples were soaked in water (1:2 ratio) for 12 h at room temperature (29 – 31 °C), and sprouted for different durations and temperatures. Significant varietal differences were observed in the IDF, SDF and TDF content of all legumes. Soaking and sprouting increased TDF and also led to considerable increase in SDF. In general, 4 – 11% of phytate phosphorus decreased during soaking, 17 – 27% during boiling and 14 – 22 % during sprouting of seeds. Reduction in IP6 content was 13 – 19% during soaking, 27 – 30% during boiling and 32 – 56% during seed sprouting. The concentrations of Ca, Mg, Fe and Zn were increased during soaking and sprouting whereas boiling decreased the Ca, Mg and Fe concentrations. They observed that the solubility of minerals was higher during soaking and sprouting than during boiling.

Sampath *et al*. (2008) studied the effect of sprouting on different cereals and pulses considering their importance as prebiotics by estimating oligosaccharides (stachyose, raffinose, maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose) contents. Sprouting of seeds for 48 h resulted in complete disappearance of stachyose and raffinose in cereals and pulses. The maltotriose content in pulses completely disappeared on sprouting but among cereals, 45.1 % and 57.3 % loss was observed in sorghum and maize, respectively, whereas complete loss was seen in other cereals tested. Arora *et al*. (2009) developed two types of food mixtures using raw and sprouted barley flour along with whey powder and tomato pulp in 2:1:1 proportion (w/w). The developed food mixtures were mixed with water, autoclaved, cooled and fermented at 37 °C for 12 h with Lactobacillus acidophilus curd containing 106 cells/ml. In sprouted and fermented food mixtures, in-vitro digestibility of protein and starch, and in-vitro availability of minerals increased 2 and 4 folds, respectively as compared to non-sprouted unprocessed food mixtures.

2.5.9 Sprouted seeds and human health

Many modifications in sprouting behave like the human digestion process and implicate an improved availability of macro- and micro-nutrients as confirmed by several in vitro and in vivo studies (Márton *et al*., 2010). Therefore, sprouted grains are a complex food matrix, where nutrients are nearly fully available, rich in various antioxidant and bioactive compounds, representing health promoting food. In the past, many health benefits have been argued by motivating the strong in vitro antioxidant potential, although the relationship between those data and the redox status measurable in vivo is very weak. Furthermore, this approach ignores other biological effects, that perhaps could be related to compounds which may not be detected by an in vitro assay, but, thanks to a good bioavailability, can activate/deactivate an oxidative-related metabolic pathway or interfere with gene regulation or with other signaling pathways (Fardet *et al*., 2008).

Nowadays, the most important preclinical and clinical studies have been focused on sprouted brown rice. Sprouted brown rice has proven strong potentials for better glycemic control, correction of dyslipidemia, amelioration of oxidative stress, reduced type 1 tissue plasminogen activator inhibitor (PAI-1), enhanced adiponectin concentration, and increased sodium potassium adenosine triphosphatase and homocysteine thiolactonase activities. These effects cannot be attributable to a single individual bioactive compound, but rather to a synergic interaction between all the bioactive compounds induced also by the sprouting process (that is, GABA, oryzanol, phenolics, dietary fibers and others), so obtaining a greater functional effect when consumed as a whole food (Jacobs and Tapsell, 2007). The effects of white rice, brown rice and sprouted brown rice in the dietary management of cardiovascular diseases have been recently investigated. In pregnant rats the exposure to high-fat diet plus sprouted brown rice until 4 weeks post-delivery, influenced metabolic outcomes in offspring of rats with underlying epigenetic changes and transcriptional implications that led to improved glucose homeostasis and reduced the risk of insulin resistance manifestations. Sprouted brown rice supplied in the diet also reduced obesity complications in high-fat diet induced-obese rats, through the improvement of lipid profiles and reduction of leptin level and white adipose tissue mass. Sprouted brown rice enhanced insulin levels, insulin receptor, glucose transporters and glucose metabolism in induced-hyperglycemia mice. In addition, in human cells, a neuro-protective effect by gene modulations has been found to be provided by sprouted brown rice. These evidences represent only a preliminary indication on the potential beneficial effects of sprouted brown rice, and more research is still required (Merendino *et al*., 2014).

2.6 Rice

Rice is a staple food of the world and being a major source of carbohydrates over half the world’s population (Ohtsubo *et al.*, 2005). Rice is in the grass family of Gramineae and related to other grass plants such as wheat, oats and barley which produce grains for food.

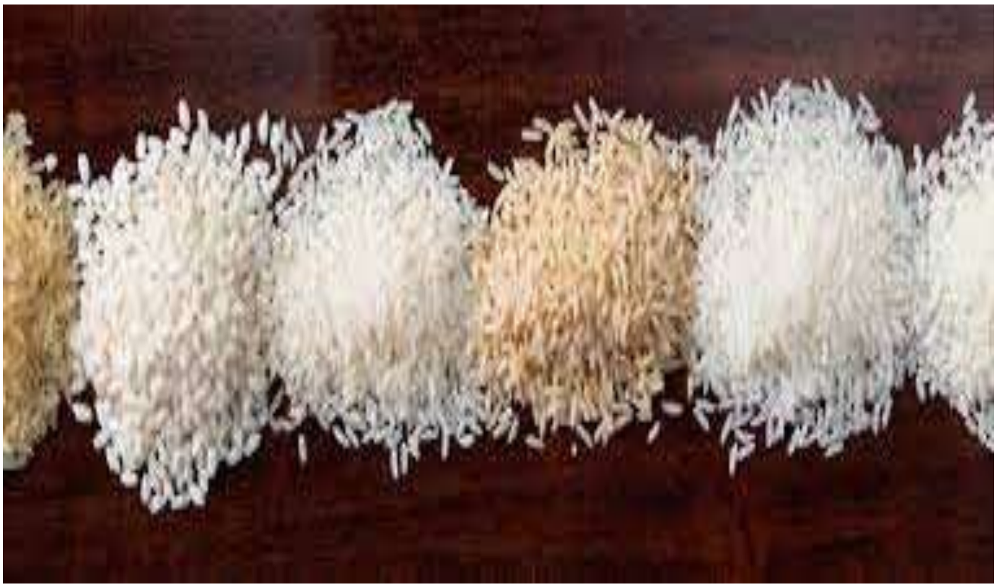

Figure 2.3: Rice seeds (Paine *et al*., 2005)

There are about 40,000 different varieties of rice worldwide. Rice contains significant amounts of thiamin, riboflavin, niacin and zinc with lesser amounts of other micronutrients. Rice contains small amount of lipids (below 5 % on dry weight basis) predominated with long chain fatty acids such as linoleic (18:2) and linolenic (18:3) acids. Rice bran is rich in these fatty acids and comprises about 15 % on dry weight basis. Long storage can degrade these unsaturated fatty acids which results in deterioration of flavor, tastiness and eating quality (Zhou *et al.*, 2003). In non-glutinous brown rice, lipid content and fatty acids composition are affected by daily mean temperature during the ripening stages. Although rice is a good source of protein, it is not a complete protein. Though in smaller amounts, it contains all of the essential amino acids required for a good health. It is better to eat unpolished (brown) rice, because the outer bran layer of the rice grain, which is removed during the milling process, is rich in fiber, iron, vitamins and minerals (Taira *et al.,* 2019).

2.6.1 Rice nutritional and health benefits

Rice is the second most widely consumed cereal in the world next to wheat. It is the staple food for two thirds of the world’s population. Over 2 billion people in Asia alone derive 80 % of their energy needs from rice, which contains 80% carbohydrates, 7 – 8 % protein, 3 % fat, and 3 % fibre (Vito *et al*., 2019). Table 2.1 below show the nutrient contents of brown rice and also comparison with other cereals and tuber crops.

Table 2.1: Proximate Compositions of Cereal and Tuber Staple Foods

Food	Moisture %	Protein (%)	Crude fat	Available carbohydrates	Dietary Fibre (g)			Crude ash (g)	Energy (kJ)	Energy (kcal)
				(%)	(%)	Water insoluble	Lignin			
Brown	14	7.3	2.2	71.1	4	2.7	0.1	1.4	1610	384
Wheat	14	10.6	1.9	61.6	10.5	7.8	0.6	1.4	1570	375
Maize	14	9.8	4.9	60.9	9	6.8	0	1.4	1660	396
Millet	14	11.5	4.7	64.6	37	2.3	0	1.5	1650	395
Sorghum	14	8.3	3.9	57.4	13.8	12.4	3	2.6	1610	384
Rye	14	8.7	1.5	60.9	13.8	8.4	14	1.8	1570	375
Oats	14	9.3	5.9	63	5.5	39	0	2.3	1640	392
Potato	77.8	2	0.1	15.4	2.5	1.9	0	1	294	70
Cassava	63.1	1	0.2	31.9	2.9	2.2	0	0.7	559	133
Yam	71.2	2	0.1	22.4	3.3	2.6	0	1	411	98

Source: (Prabha *et al*., 2018)

2.6.2 Rice fermentation

2.6.2.1 Biochemical transformation during rice fermentation

Biochemically, four different types of fermentation processes are known to take place, namely, alcoholic, lactic acid, acetic acid, and alkali fermentation. Alcoholic fermentation results in the production of ethanol by yeast (as in wine and beer). Lactic acid fermentation (homofermentative or heterofermentative) is mainly carried out by lactic acid-producing bacteria (LAB). Under excess aeration, Acetobacter produces acetic acid from alcohol. Alkali fermentation occurs in the case of fish and seeds, protein-rich food constituents (Ray and Didier, 2014).

Rice-based fermentation involves either acidic or alcoholic fermentation or both consecutively. In the beginning, the process includes pretreatment of rice grains, such as soaking, grinding, or boiling, which generally relaxes the compact structure of starch and simultaneously dilutes the contents of antinutrient components. The extended soaking in some food preparations initiates the sprouting process by activating various hydrolytic enzymes, which relax the starch compactness by exo- and endo-cleaving pathways (Ghosh *et al*., 2015). For the preparation of rice batter (mixed with pulses), microbial fermentation leads to the formation of carbon dioxide and other gases inside the batter and makes the food spongy. The degree of fermentation depends on time, which again determines food taste, texture, appearance, aroma, and so on. Rice beer preparation involves a three-step nonsynchronized fermentation process. Initially, amylolytic molds are grown, which contribute to the saccharification and liquefaction of rice (Ghosh *et al*., 2015). It appears that starch is first split into limit dextrins by the action of α-amylase, and later by the action of glucoamylase these fragmented oligosaccharides are hydrolyzed into glucose. Thereby, rice is decomposed and creates an anaerobic environment. This condition favors the growth of LAB and Bifidobacterium, and apart from lactic and acetic acid, they also produce different hydrolytic enzymes (catalyzed both nutrients and antinutrients) and various metabolites. Later, alcohol-producing yeast (Saccharomyces cerevisiae) is grown, which enriches the ferment with various vitamins, amino acids, etc., and aids specific flavor and aroma (Ghosh *et al.*, 2015). During fermentation, synchronized participation of different groups of symbiotic aerobes and anaerobes that float or embed within the matrix of cereal completely decomposes it into a simpler form for rapid assimilation through the gastrointestinal tract and favors maximum energy extraction. Thus, fermented beverages confer more health promoting attributes than solid/semisolid fermented foods (Ghosh *et al.*, 2015).

2.6.3 Functional metabolites in rice-based fermented foods

According to Roberfroid (2000), a food product can be made functional by eliminating a component known to produce a deleterious effect when consumed (an allergenic protein, lactose, phenylalanine), by increasing the concentration of a component naturally present in food to a point at which it will induce predicted effects (fortification with a micronutrients), increasing the concentration of a nutritive component to a level known to produce beneficial effects, adding a component that is not normally present in most food and is not necessarily a macronutrient or micronutrient, but for which beneficial effects have been shown (non vitamin antioxidants or prebiotic fructans), replacing a component, usually a macronutrient, the intake of which usually causes deleterious effects, increasing bioavailability or stability of a component known to produce a functional effect or to alleviate the disease risk potential of the food. Being the largest choice of food ingredient globally, rice has been found to contain more than

5,000 small metabolites (Kind *et al*., 2009). The processing of rice into various edible food items could change the profile of metabolites.

In a separate study, more than 3,000 small metabolites only have been detected after the cooking of rice. There are large numbers of evidences indicating the bioenrichment of rice with different reactive metabolites occurring during fermentation. Considering these types of scattered evidences, a scientific link or correlation is very essential to focus on the specific metabolic pathway. Thus, there is huge scope for the incorporation of systems biological approach to reveal the nutritionally important rice metabolites improves in various rice-based fermented foods and beverages. The changes of metabolite composition in the final rice-based fermented products are related with the participating microbiota and nature of fermentation (Ghosh *et al*., 2015). Rural folk unknowingly used microbes in primitive ways for the preparation of different types of food, which were enriched with unexplored metabolites, but these serve as major sources of energy, nutrients, and health-beneficial components. Most of these metabolites exert various health impacts, demonstrate protective activities over human diseases, and also manifest beneficial effects on the immune system (Fitzgerald *et al*., 2009). After fermentation, rice becomes enriched with various metabolites, some notable examples like phenolics (monophenols and polyphenols), flavons (Mono-C-glycosides, malonylated O-hexosides, O-glycosides), vitamin E (tocopherols and tocotrienols), phytosterols, linolenic acid, anthocyanins, proanthocyanidins and g-oryzanol.

2.6.4 Sprouted brown rice (SBR)

Consumption of brown rice became popular in Japan back in the 1970’s because of rich fiber and other nutrients contained in the brown rice. However, the popularity did not last long due to the fact that brown rice had to be cooked in the pressure cooker and was still hard to chew and less tasty. Sprouted brown rice (SBR) overcame the problem which can be cooked in an ordinary rice cooker and is soft enough to chew even for children (Roohinejad *et al.*, 2011). SBR has a mellow flavor and a soft mouth feel. Further, SBR is much more nutritious and has numerous health benefits. SBR is also called as 'germinated brown rice'. The process of sprouting enhances the bio-availability of nutrients by neutralizing phytic acid. Consumption of unsprouted grains can lead to poor absorption to nutrients in the grain. The incompletely digested proteins can irritate the intestines, leading to inflammation and allergic reactions. Neutralizing the phytic acid, releasing the proteins, vitamins, and enzymes allow these important nutrients to be absorbed during digestion. SBR is different from normal brown rice in that it has undergone the process of sprouting; more specifically, the rice embryo is sprouted under suitable environmental conditions (Roohinejad *et al.*, 2011).

Generally, brown rice can be sprouted by soaking it in warm water of 35 – 40 °C for about 10 – 12 hours, draining water and keeping in moist condition for 20 – 24 hours, and during soaking period, changing the water every 3 – 4 hours to prevent fermentation (which usually produces undesirable odor) and to maintain consistent water temperature. The result yields a 0.5 – 1 mm long sprout from the brown rice grain; at this stage nutrient accumulation in the grain is maximal. Manufactured SBR is mostly sold in dried form (the drying does not affect the superior nutritional value accumulated from sprouting), which looks very similar to ordinary brown rice. The effect of the drying process is to prolong shelf life.

Furthermore, the sprouting of brown rice (thereby making SBR) is necessary for enhancing nutrients required to maintain and promote health (Hiroshi, 2005). SBR is evaluated as a functional food because it is good in digestion and absorption, and contains more nutrients such as (GABA) and ferulic acid as compared to ordinary brown rice. Dried SBR offers an excellent appearance, improved shelf life and handling ease. Unlike white rice, SBR provides more sweetness, excellent taste, has better texture and is easier to cook (Hiroshi, 2005). The brown rice is soaked for one or two nights depending on the ambient temperature and then sprouted. This process changes the internal minerals and the brown rice becomes more nutritious, easier to chew and tastier. It has been reported that the SBR may enhance brain functions and reduce level of lipids, or fats, in the blood.

2.6.4.1 Physico-chemical changes in SBR

Various types of analyses on SBR conducted in Japan indicated that during the process of sprouting, nutrients in the brown rice change drastically. Kayahara *et al*., (2001) showed that, not only existing nutrients are increased but new components are also released from the inner change due to sprouting. The nutrients which increase significantly include GABA, lysine, vitamin E, dietary fiber, niacin, magnesium, vitamin B_1, and vitamin B_6. The other nutrients that increase in SBR are inositols, ferulic acid, phytic acid, tocotrienols, potassium, zinc, g-oryzanol, and prolylendopeptidase inhibitor. In particular, the amount of GABA in SBR is observed to be ten times more as compared to milled white rice and two times more than that of brown rice. Further, they found that SBR contains less calories and sugar than that in milled rice. Trachoo *et al*., (2006) found that sprouting of rice grains increased many nutrients such as vitamin B, reducing sugar, and total protein contents of SBR were higher than those of brown rice and white rice.

2.6.4.2 Health benefits of SBR

Nutrition of sprouted grains has been studied since decades ago. Kayahara *et al*., (2001) concluded that continuous intake of SBR is good for preventing headache, relieving constipation, preventing cancer of colon, regulating blood sugar level and preventing heart disease. Okada *et al*., (2000) reported that intake of GABA suppressed blood pressure and improved sleeplessness, and autonomic disorder observed during the menopausal or presenile period. SBR helps in preventing Alzheimer’s disease, due to its increased GABA content. Kayahara *et al*., (2001) showed that the brown rice sprouts contain a potent inhibitor of an enzyme called protyl endopetidase, which is implicated in Alzheimer’s disease. Ito *et al*., (2005) reported that intake of SBR instead of white rice is effective for the control of postprandial blood glucose concentration without increasing the insulin secretion in subjects with hyperglycemia. Hiroshi (2005) showed that besides containing other useful components, SBR mainly contains two active components viz. GABA, a neurotransmitter which is abounding in brain and spinal cord, and dietary fiber which activates the peristalsis of intestine. It also contains considerable phytic acid with a powerful anticancer activity and a prolyl endopeptidase activity inhibitor related to the metabolism of peptide. Chikako *et al*., (2005) reported that the protease activity in SBR was increased 1.5 times after sprouting. They suggested that decrease in soluble proteins and allergens was induced in part by proteolytic degradation and two abundant allergens were degraded in a different manner and probably by different protease in the grains during sprouting.

2.6.5 Uses of SBR

In Japan, people in the ancient era may have been eating soaked brown rice. Komatsuzaki *et al*., (2003) reported effects of soaking and gaseous phase sprout processing on the GABA content in SBR. Their study established a processing method that can be used to accumulate high concentrations of GABA. (Ohtsubo *et al*. 2005) explained that in sprouted grains, starch, non-starch polysaccharides and proteins get partially hydrolyzed to sugars, oligosaccharides and amino acids, which also occur in rice. This phenomenon will create bio-functional substances and improvement in palatable texture of cereal grains. In addition, sterilization treatment of SBR a standard process for commercialized SBR products to reduce micro-organisms results in an increased amount of glutamic acid, alanine, and glycerin which produce a sweeter and more enhanced flavor as compared to the ordinary brown rice (Ohtsubo *et al*. 2005). Watanabe *et al*., (2004) showed that the substitution of brown rice or SBR for wheat flour lowered specific volume of bread more than the control bread without brown rice (BR) or SBR with increasing amounts of substitution. The improving effect was more obvious for 10 or 20 % SBR than for BR. Of the bio-functional components in BR and SBR breads, GABA was unexpectedly decomposed from the final bread. Therefore, SBR would improve the bread quality when substituted for wheat flour. Mamiya *et al*., (2006) investigated the antidepressant-like effects of SBR and polished rice pellets in comparison with control (AIN-93G) pellets in the forced swimming test and they learned helplessness paradigm in mice. The immobility time on the second day of the forced swimming test was shorter in mice fed with polished rice or SBR pellets than in mice fed with control pellets. These results suggested that the increase of 5-hydroxytryptophan levels in the mouse frontal cortex contributes to the antidepressant-like effects of SBR pellets. GABA, glutamine, and glycerin are amino acids that form certain neurotransmitters of the brain which are inhibitory and have ability to reduce the transmittal of stress, anxiety, grief or depression related messages from the limbic system to the cortex. With a reduction in these messages, the emotional responses will be dampened and make a person feel more relaxed with a better sense of well-being. Shigeko *et al*., (2007) showed that the SBR have beneficial effects on psychosomatic health and relationship between lactation and SBR has attracted interest in terms of mental health and immunity.

2.6.6 Gamma-aminobutyric acid and its effects in SBR

The reason behind the popularity of SBR among consumers is the significant increase in GABA, a four-carbon nonprotein amino acid, which is an inhibitory neurotransmitter that have the following benefits: promotes fat loss by the stimulation of the production of human growth hormone; increases the sleep cycle giving deeper rest; boosts the immune system; lowers blood pressure; inhibits development of cancer cells; assists the treatment of anxiety disorders (Ito and Ishikawa 2004). This free amino acid has also effects on accelerating metabolism in brain, preventing autonomic disorders during presenile ormenopausal period, and relieving insomnia (Komatsuzaki *et al*. 2003). Further, Ito and Ishikawa (2004) suggested that GABA might have preventive effects on Alzheimer's disease, or help lessen symptoms experienced from this disease and other cerebral related disorders, such as amnesia and dementia.

Apart from GABA prevalent in SBR, many food scientists are now also focusing on Gamma-oryzanol, ferulic acid, which also increases from the sprouting process. The substance has an anti-oxidative effect that can prevent skin aging and regulate cholesterol levels (Kayahara *et al*. 2001). However, the increase is not as significant as GABA and its function has not been proven to be as diverse as GABA.

2.7 Sesame Seeds

Sesame seeds (Sesamum indicum) are tiny, flat oval seeds with a nutty taste. It is an important oil seed believed to have originated from tropical Africa with the greatest diversity (RMRDC, 2004). Sesame seed is a staple food among many ethnic groups in Nigeria and it is cultivated in most areas of the middle belt and some northern states of Nigeria (Olanyanju *et al.*, 2006). Sesame is an important source of oil (44 - 52.5 %), protein (18 - 23.5 %), carbohydrate (13 %). The seeds are rich in mono-unsaturated fatty acid (oleic acid) and equally rich sources of many minerals such as calcium, phosphorus, manganese, zinc, magnesium and potassium which play vital roles in the body (Makinde and Akinoso, 2013). The seeds are consumed fresh, dried or blended with sugar. It is also used as a paste in some local soups. It is also consumed for its medicinal qualities. Among all the oil seed proteins, sesame protein is the most nutritious as it is a rich source of methionine (sulphur containing amino acid and tryptophan. Because of its greater and varied utility, it is considered as the Queen of oilseeds. Processed sesame seeds are used in baked products; cake, hamburger, buns, cookies, confectionery purposes and many snack foods (Nagaraj, 2009).

Figure 2.4: Sesame Plant Showing Numerous Flowers and Seeds (Langham, 2008)

The nutritional value of foods depends on their nutrient content and the bioavailability of these nutrients. The nutritional quality of sesame seed and its products can be enhanced by roasting, boiling and de-fatting prior to consumption. Sesame is commercialized in a number of forms. Sesame seeds can also be consumed directly as a highly nutritious foodstuff (Naturland, 2002). Various processing technologies have helped in transforming food ingredients into healthier products with maximum nutritional value to ensure nutrient security of the population in developing countries (Kumar, 2010). Such techniques include Fermentation, boiling, roasting and Sprouting. The most common domestic processing methods include ordinary cooking (boiling) and roasting (Hassan, 2011). The widespread and long-standing tribute to sesame lies in its high oil content, nutritious protein, and savoury roasted flavour. Cooking has been reported to improve the nutritional and functional properties of the plant seeds (Yagoub & Abdalla, 2007). Boiling and Roasting can also reduce malnutrition by making micronutrients available for easy absorption; hence, increasing the utilization of sesame seeds. Although exists numerous studies about the nutritional characteristics of sesame seeds, there is little information on nutrient composition in value added products formulated with fermented, boiled, roasted and sprouted sesame seed flour. Industrial processing and utilization of sesame have not been fully developed in Nigeria as its utilization is restricted to producing regions; for the most part, the surplus crop is commercialized, bulked and exported.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Sampling of materials

The paddy rice (FARO 52) and sesame variety (NCRIBEN 04E) was obtained from National Cereals Research Institute (NCRI), Badeggi, Niger State.

The rice samples were manually cleaned to remove shaft before milling to obtain brown rice whereas the sesame samples were kept until required.

3.1.2 Chemicals and reagents

All chemicals and reagents used were of analytical grade, products of British Drug House (BDH) and Mayer/Baker grades. The chemicals used include sodium hydroxide, hydrochloric acid, sulphuric acid, perchloric acid, cupric sulphate and ammonium thiocyanate among others.

3.1.3 Equipments

The equipments used in this study include attrition mill (RM 100 model), amino acid analyzer (Beckman system 6300 Model), rice dehuller (satake Tokyo M3), extruder (Duisburg, DCE-330 model), muffle furnace (carbolite, Bamford, S302AU) among others.

3.2 Methods

3.2.1 Fermentation of rice

A modified method of Jeygowri *et al.,* (2015) was adopted for the fermentation of the rice samples. Brown rice was soaked in warm water for 30 minutes to obtain uniform hydration. Fermentation was carried out by soaking brown rice in sterile distilled water (24 hours) at an ambient temperature (37 ± 2 ℃). After the fermentation, the water in the container was drained and the fermented brown rice was air dried before transferring into an oven where the moisture content was reduced to 12 % before milling into flour and sieved through a laboratory sieve of 80 mesh size, packaged in polyethylene bags until required for further experiment.

3.2.2 Fermentation of sesame seed

The sesame seeds were dehulled and fermented using the method described by Akusu *et al*., (2019) with little modifications. The seeds were boiled in hot water for 6 hours and cooled. The cooked seeds were placed in a plastic container with a tight lid and sealed. The samples were allowed to ferment at room temperature (37 ± 2 ℃) for 7 days and oven dried at 105 °C for 12 h to bring an end to fermentation. The seeds were defatted using hydraulic press and milled to obtain fermented sesame flour and stored in a glass container.

3.2.3 Sprouting of rice and sesame seeds

The seeds were germinated as described by Akusu *et al*, (2019). The seeds were sorted to remove stones and other extraneous materials. They were thereafter soaked for 2 h to achieve hydration then rinsed, drained and spread thinly on jute sack for germination to take place. The germination process was closely monitored to prevent discontinuity of germination and mould growth which was achieved by constant wetting and intermittent uniform spreading of the germinating seedlings. Sprouting was carried out for 7 days. The sprouted seedlings were thoroughly rinsed with water, drained, derooted, dried in a hot air oven set at 60 ºC for 6 h and the sesame seeds were defatted using hydraulic press before milling using a laboratory blender to pass through a 0.5 mm sieve and stored in plastic bags until required for further analysis.

3.2.4 Composite flour formulation

Five composite flour blends were formulated from fermented and sprouted samples and coded as: $FR_{90}S_{10}$, $FR_{80}S_{20}$, $FR_{70}S_{30}$, $FR_{60}S_{40}$ $FR_{50}S_{50}$ and $SR_{90}S_{10}$, $SR_{80}S_{20}$, $SR_{70}S_{30}$, $SR_{60}S_{40}$ and $SR_{50}S_{50}$. They were prepared by mixing varying proportions of fermented rice-defatted sesame flour for both fermented and sprouted samples. The control was 100% untreated rice (R_{100}) as shown in table 3.1.

Table 3.1: Percentage Composition for Rice and Sesame Composite Flours

Composite	Rice Flour	Sesame Flour
$FR_{90}S_{10}$	90	10
$FR_{80}S_{20}$	80	20
$FR_{70}S_{30}$	70	30
$FR_{60}S_{40}$	60	40
$FR_{50}S_{50}$	50	50
$SR_{90}S_{10}$	90	10
$SR_{80}S_{20}$	80	20
$SR_{70}S_{30}$	70	30
$SR_{60}S_{40}$	60	40
$SR_{50}S_{50}$	50	50
Control (R_{100})	100	---

FRS: Fermented Rice-Sesame
SRS: Sprouted Rice-Sesame
R_{100}: Untreated rice

3.2.5 Extrusion cooking experiment

The different rice-sesame formulations were subjected to extrusion cooking using a small scale laboratory single screw extruder. The moisture content of the flour was determined and adjusted to 25 % according to the methods described by Anuonye *et al.*, (2012). Feeds were manually introduced at a speed of 30 rpm which insure that the flight of the screw was filled and avoiding accumulation of feed in the hopper. Desired barrel temperature (120 ℃) was maintained by in-build thermostat and a temperature control unit. Experimental samples were collected as steady state was achieved. The extrudates were dried overnight after extrusion at 60 °C in an oven and crushed using a small scale laboratory blender into powder forms then stored in a desiccator till required for analysis and sensory evaluation.

3.2.6 Analyses of extrudates

3.2.6.1 Determination of proximate composition

3.2.6.1.1 Moisture content

The moisture content of the samples was determined in triplicate by hot-air oven method according to AOAC (2010). Each sample (2 g) was weighed into thoroughly washed previously dried dishes and placed in an oven at 100 – 102 °C for 2 – 3 hours. The dishes were cooled in a dessicator and weighed. Drying, cooling and weighing were continued until a constant weight was obtained. Thereafter, the dry weight of the sample plus crucible was recorded and used to calculate moisture content with the expression.

Moisture content (%) = A - B x 100/C

Where C = Sample weight in g

A = Weight of dish + sample before drying

B = Weight of dish + sample after drying

A - B = Loss in weight of sample after drying

3.2.6.1.2 Crude protein

This was determined by Kjeldahl method as described by AOAC (2010). Each sample (1 g) was weighed into a Kjeldahl flask and 3.0 g of hydrated cupric sulphate (catalyst), twenty (20) ml of sodium sulphate solution and 1.0 ml of concentrated sulphuric acid (H_2SO_4) was added to sample in the flask. The flask was clamped and heated until the solution became colourless. The clear solution was cooled and diluted with distilled water to make up the volume to 100 ml. Ten (10) ml of the digest was mixed with 5 ml of 40 % sodium hydroxide solution in a distillation flask and distilled to release ammonia which was titrated with 0.1 ml hydrochloric acid (HCl). The titre value or end point at which the colour changes from green to pink was noted and the crude protein was calculated using the expression.

Crude protein (%) = 14.01 x 6.25 x 25 x T x 100/W x 10

Where:

N = Normality of HCl, in moles/1000 ml (0.1 N)

6.25 = Conversion factor

VF = Total volume of the digest= 100 ml

T = Titre Value

10 = Aliquot Volume distilled

W = Weight of the sample digested

3.2.6.1.3 Crude fibre

The crude fibre content of the sample was determined using the method of AOAC (2010). The whole sample will first be defatted with n-hexane, dried and then 2 g weighed (W_1) into a beaker, boiled for 30 minutes with 100 ml of H_2SO_4 then filtered through a filter paper. The residue was washed with boiling water until the washing was no longer acidic. The washed residue was boiled for another 30 minutes with 100 ml of 0.02 M NaOH solution, filtered and washed with hot water for 3 minutes. The residue was transferred into a previously ignited, cooled and weighed crucible and dried in the oven for 1 h. The crucible with its content was cooled in a dessicator and then weighed

(W_2) using digital balance. The cooled sample will then be ignited in a muffle furnace at 600 ºC for 3 h, cooled and weighed (W_3) using digital balance.

The percentage crude fibre was calculated with the expression.

% Crude fibre = $W_2 – W_3$ x $100/W_1$

Where W_1 = Initial weight of the sample

W_2 = Weight of the sample + crucible

W_3 = Weight of the sample + crucible after ashing

3.2.6.1.4 Crude fat

The Soxhlet extraction method (AOAC, 2010) was used in determining fat content of the sample. The sample (2 g) was weighed in a digital balance and put in a cellulose thimble. The thimble and its content was placed in the extraction tube of the Soxhlet apparatus. A weighed round bottom flask is to be filled to about three quarter (3/4) of its volume with petroleum ether fitted to the extraction tube and set on a heating mantle. The sample was refluxed for 6 - 8 h after which the solvent (petroleum ether) was recovered and the extracted oil in the flask dried in the oven at 80 ℃ for 30 minutes to remove solvent traces, cooled in a dessicator and finally weighed using digital balance. The fat content was expressed as a percentage of the raw material. The difference in the weight of empty flask and the flask with oil gave the oil content which was calculated with the expressions:

Fat (%) = A – C x 100/B

Where: A = Weight of flask + oil

B = Weight of sample in g

C = Weight of empty flask

3.2.6.1.5 Ash content

The ash content was determined using the method of AOAC (2010). Each sample (2 g) is to be weighed into a silica dish previously washed heated to about 600 ℃ and cooled in a desiccator then weighed using digital weighing balance. The silica dish and the sample was heated in a muffle furnace at about 700 ℃. This temperature is to be maintained until whitish-grey coloured ash is obtained indicating that all the organic matter in the product has been destroyed. The dish is to be cooled in a dessicator and weighed using a digital balance. The percentage ash content is to be calculated using this expression:

% Ash = B- A x 100/C

Where A = Weight of crucible

B = Weight of crucible + ash

C = Weight of original sample

3.2.6.1.6 Carbohydrate content

The carbohydrate content was determined by difference. Sum of all the proximate components was subtracted from hundred (100). The balance was assumed to be carbohydrate.

% Carbohydrate = 100 - (% protein, fat, fibre, ash and moisture).

3.2.7 Amino acid determination

The amino acid profile of the extruded samples was determined using the methods described by AOAC (2012). The samples were first dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Technicon sequential Multi-Sample Amino Acid Analyzer (TSM).

3.2.8 Mineral content determination

Ash was determined by combustion of the sample in a muffle furnace at 550 °C for 12 h (AOAC 2012). The ash was quantitatively transferred into 150 ml beakers. To the ashes in the beakers, 10 ml of distilled water, 10 ml of concentrated perchloric acid, 10 ml of concentrated hydrochloric acid and 10 ml of concentrated trioxonitrate (v) acid was added. The beakers were covered and heated at a temperature of 120 °C on a hot plate until the emission of brown fumes stopped. The sides of the beakers were rinsed with distilled water and then heated further to concentrate the solution to 5 ml. the beakers will then be removed and cooled before adding 10 ml of concentrated HNO_3. To the solution, 5 ml of distilled water was added and boiled for 5 minutes. The solutions will then be filtered into 100 ml standard volumetric flask and made up to the mark with distilled water. These diluents were aspirated into atomic absorption spectrophotometer (AAS) through the suction tube. Each of the trace mineral elements was read at their respective wavelengths with their respective hollow cathode lamps using appropriate fuel and oxidant combination. The metals determined were; Ca, Mg, K, Na, P, Fe and Zn. (AOAC 2012).

3.2.9 Anti-nutrient content determination

3.2.9.1 Phytate

The standard method of Soetan (2012) was used. The powdered sample (4 g) was soaked in 100 cm^3 of 2 % HCl v/v for 3 h and then filtered. To the 25 cm^3 of the filtrate, 5 cm^3 of 0.3 % NH_4SCN and 53 cm^3 of distilled water was added and mixed. The mixture was then titrated against 0.001 M standard $FeCl_3$ solution until a brownish yellow colour persists for 5 seconds. Phytin phosphorus (1 cm^3 = 1.19 mg phytin phosphorus) was determined and the phytic acid content was calculated by multiplying the value of the phytin phosphorus by 3.55.

3.2.9.2 Oxalic acid

Two grams of the powdered sample was put into a 250 cm^3 conical flask containing 190 cm^3 of distilled water and 10 cm^3 of 6 M HCl (v/v). The mixture was diluted for 1 h in a boiling water bath. After cooling, the mixture was then filtered. 50 cm^3 aliquot of the sample was placed into a beaker and 20 cm^3 of 6 M HCl (v/v) was added. The mixture was evaporated to about half of its volume and then filtered. The residue was washed several times with distilled water, and 3 drops of methyl orange indicator was added to 25 cm^3 of the filtrate and titrated against 0.1 M $KMnO_4$ w/v solution till a faint pink colour appeared and persisted for 30 seconds (Day and Underwood, 1986).

Oxalate (mg) = Titre value X 0.0045

3.2.9.3 Tannin content

Tannin content of the extrudate was determined by the method described by Price and Butler (1977). Powdered sample (0.2 g) was weighed into Erlenmeyer flask, and 10 ml of 4 % HCl in methanol was pippeted into the flask. The flask was closed with parapilus and shaken for 20 minutes on a wrist actron shaker. The extract (1 ml) was pippeted and 1 ml of 1 % vanillin and 0.5 ml of concentrated HCl was added.

Five test tubes were labelled I, II, III, IV and V to prepare the standard solutions. Into the five test tubes, 0.1, 0.3, 0.5, 0.7 and 1.0 ml of phenol reagent was added respectively. The test tubes were made up to 1 ml with methanol (8 % HCl in methanol). 1.0 ml of 1 % vanillin and 0.5 ml conc. HCl was added to the tubes and made up to 5.5 ml with 4 % HCl in methanol. Blank sample was prepared by using 5 ml of 4 % HCl in methanol. The absorbance of the standard solutions, sample extract and blank sample was read using a spectrophotometer at 500 nm for 20 minutes after incubation.

Calculation:

$$Cu = \frac{Au \times Cstd}{Astd} = mg/g$$

Where

Au = Absorbance of unknown.

Astd = Absorbance of standard.

Cu = Concentration of unknown.

Cstd = Concentration of standard

3.2.10 Determination of vitamin B content

Vitamins B_1, B_2, B_6 and B_{12} were determined using spectrophotometric method (AOAC, 2011). Thiamine content was determined by weighing 0.5 g of the sample and adding 30 ml dichloroethane and 30 ml of 30 % HCl (ratio1:1). Then 50 ml ammonium hydroxide solution was added. The solution was then filtered using whatman filter paper. Then the absorbance was read on a spectrophotometer at 415 nm. Riboflavin content was determined by weighing 1 g of the sample and adding 50 ml of 50 % methanol and 50 ml of 17 % sodium carbonate. This is the extraction. Then the absorbance was read on a spectrophotometer at a wavelength of 415 nm. Vitamin B_6 content was determined by weighing 0.1 g of sample into a conical flask and 2 ml was extracted with MeOH-NaOH and 1 ml supernatant was pipetted after spinning, 20 microlitre of 0.5 % $KMnO_4$ was added and absorbance was taken at 420 nm.

Vitamin B_{12} content of extrudates were determined by weighing 0.1 g of sample and 5 ml of 3 % Na_2HPo_4 was added, spinned and filtered 1 ml of the supernatant was pipetted and 0.4 ml of 0.4 % $KmnO_4$ was added to the supernatant, 0.4 ml of 2 % $NaNO_3$, 0.4 ml of 5 % H_2SO_4, 0.4 ml of 5 % EDTA and 100 microlitre of 0.1 % Azo dye were added to the mixture and allowed to stand for 100 minutes and the absorbance was read at 550 nm.

3.2.11 Determination of functional properties

Functional properties such as bulk density, water absorption capacity, swelling capacity, swelling power, were determined following the methods of Onwuka (2005) while water solubility index was determined following the methods described by Nahemiah *et al.*, (2017).

3.2.11.1 Bulk density

The bulk densities (BD) of the samples were determined as previously reported by Onwuka (2005). About 10 g of the sample was weighed into 50 mL graduated measuring cylinder. The sample was packed by gently tapping the cylinder from a height of 5 cm. The volume of the sample was recorded and packed bulk density was calculated as ratio of weight of sample and volume of the sample after tapping. Loose bulk density was determined by filling the sample into the measuring cylinder to mark (50 mL), and the content was weighed.

$$\text{Bulk density (g/mL)} = \frac{Weight\ of\ sample}{Volume\ of\ sample\ after\ taping} \quad \text{(packed bulk density)}$$

Bulk density (g/mL) = $\frac{Weight\ of\ sample}{Volume\ of\ sample\ before\ taping}$ (loose bulk density)

3.2.11.2 Water absorption capacity (WAC)

Water absorption capacity of each sample was determined by the method of Onwuka (2005). Each sample (1 g) is to be weighed into a dry centrifuge tube. Distilled water is mixed with the sample to make up to 10 ml dispersion. It is to be centrifuged at 3500 rpm for 15 minutes. The supernatant is then decanted while the tube with the content was reweighed. The gain in mass was calculated as the water absorption capacity of the sample.

WAC (g/g) = weight of sediment – weight of sample.

3.2.11.3 Water solubility index (WSI)

Water solubility index was determined according to the method described by Nahemiah *et al*., (2017). Extruded foods were grounded and sieved through a 210 µm sieve and 1.0g taken and placed in a centrifuge tube and 10 ml distilled water was added. After standing for 15 minutes and shaking at every 5 minutes interval, the samples were centrifuged for 15 minutes at 100 rpm. The supernatant was decanted and the weight gain in the gel was recorded. The supernatants were dried overnight at 90 ˚C and WSI was determined as weight of dried supernatant divided by the weight of dry sample.

WSI (g) = $\frac{Weight\ of\ dissolved\ solid \in supernatant}{weight\ of\ dry\ solid}$

3.2.11.4 Swelling capacity/ index (SC/SI)

The method of Onwuka (2005) was used to determine the swelling capacity. Each sample (3 g) was weighed into clean dry and graduated 50 ml cylinders. The samples were gently levelled and the volume was taken before the addition of 30 ml distilled water. The cylinder was swirled manually and allowed to stand for 60 minutes while the changes in the volume (swelling) were recorded. The swelling capacity of each sample was calculated as the final volume divided by the initial volume of the sample in the cylinder.

Thus, initial volume = V_1 cm^3

Final volume = V_2 cm^3

Difference in volume = $V_2 – V_1 = \Delta V$ cm^3

% increase in volume =$\Delta v/v_1 \times 100$

Thus, % increase was used as a measure of swelling capacity.

3.2.12 Sensory evaluation of extrudates

Extrudates from fermented and sprouted rice and sesame seed composite flours were subjected to sensory evaluation method described by Giami and Barber (2004). They were evaluated for Appearance, taste, aroma, color, mouthfeel, aftertaste and overall acceptability. The ratings were on a 9-point hedonic scale ranging from 9 (like extremely) to 1 (dislike extremely) as described by Iwe (2010). The mean scores were analyzed using analysis of variance (ANOVA).

3.3 Data Analysis

The results obtained from the various analyses were subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 16.0. All data were analyzed at a 95 % confidence interval and values were considered statistically significant at $P < 0.05$.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Proximate composition of fermented and sprouted rice - sesame composites

The proximate composition of the formulated rice-sesame blends is shown in table 4.1. The result of the proximate analysis carried out on the fermented and sprouted rice-sesame composites showed considerable and significant variability in the mean moisture, ash, crude protein, crude fibre, crude fat and carbohydrate content. Significantly ($P < 0.05$) higher moisture contents of 10.62 ± 0.01 % and 11.70 ± 0.14 % were obtained in $FR_{50}S_{50}$ and $SR_{50}S_{50}$ and the lowest values of 8.72 ± 0.01 % and 10.72 ± 0.01 % were found in $FR_{90}S_{10}$ and $SR_{50}S_{50}$ for the fermented and sprouted seed blends respectively. The ash content of the composites was significantly ($P < 0.05$) higher in $FR_{50}S_{50}$ (1.89 ± 0.03 %) for the fermented seed blends while $SR_{60}S_{40}$ (1.96 ± 0.01 %) and $SR_{50}S_{50}$ (1.99 ± 0.03 %) were not statistically different for the sprouted seed blends. Lower ash contents were recorded in $FR_{90}S_{10}$ (0.61 ± 0.01 %) and $SR_{90}S_{10}$ (1.08 ± 0.01 %) for the fermented and sprouted rice/sesame composites respectively. Similarly, the fat content was observed to be statistically ($P < 0.05$) higher in $FR_{50}S_{50}$ (15.42 ± 0.01 %) and $SR_{50}S_{50}$ (11.52 ± 0.01 %), and lower in $FR_{90}S_{10}$ (6.92 ± 0.15 %) and $SR_{90}S_{10}$ (4.51 ± 0.14 %) for the fermented and sprouted seed blends, when the sesame composition increased respectively. With regard to the protein content, significant increase was observed with increased sesame addition with the higher values seen in $FR_{50}S_{50}$ (24.39 ± 0.04 %) and $SR_{50}S_{50}$ (25.35 ± 0.04 %), while the lowest values were recorded in $FR_{90}S_{10}$ (10.26 ± 0.0 %) and $SR_{90}S_{10}$ (11.60 ± 0.14 %) for the fermented and sprouted seed blends respectively. The result of fibre content also indicated that formulations corresponding to equal proportions ($FR_{50}S_{50}$ and $SR_{50}S_{50}$) of the fermented and sprouted seed blends had higher crude fibre (1.25 ± 0.01 % and 1.31 ± 0.01 %) while the lowest values were recorded in $FR_{90}S_{10}$ (0.40 ± 0.01 %) and $SR_{90}S_{10}$ (0.71 ± 0.01 %). The carbohydrate content was statistically higher in R_{100} (85.16 ± 0.04 %) than the highest values recorded in $FR_{90}S_{10}$ (73.08 ± 0.04 %) and $SR_{90}S_{10}$ (71.38 ± 0.02 %) for the fermented and sprouted seed composites respectively. The calorific values (Kcal/100 g) were found to be statistically ($P < 0.05$) higher in $FR_{50}S_{50}$ (422.06 ± 0.20 Kcal/100 g) and $SR_{50}S_{50}$ (397.76 ± 0.14 Kcal/100 g) while the least values were seen in $FR_{90}S_{10}$ (395.64 ± 0.04 Kal/100 g) and $SR_{90}S_{10}$ (372.51 ± 0.04 Kcal/100 g) for the fermented and sprouted seed blends respectively, while the overall lowest caloric value was recorded in R_{100} (370.08 ± 0.01).

Table 4.1: Proximate composition of fermented and sprouted rice-sesame composites

Sample	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Fibre (%)	CHO (%)	Energy Value (Kcal)
FR$_{90}$S$_{10}$	8.72±0.01^{d}	0.61±0.01^{d}	6.92±0.15^{e}	10.26±0.01^{e}	0.40±0.01^{c}	73.08±0.04^{a}	395.64±0.04^{e}
FR$_{80}$S$_{20}$	9.39±0.02^{c}	1.32±0.14^{c}	9.10±0.08^{d}	12.91±0.14^{d}	0.87±0.02^{b}	66.41±0.01^{b}	399.18±0.12^{d}
FR$_{70}$S$_{30}$	9.48±0.01^{c}	1.35±0.01^{c}	12.17±0.01^{c}	15.38±0.01^{c}	0.89±0.04^{b}	60.73±0.01^{c}	413.97±0.02^{c}
FR$_{60}$S$_{40}$	9.70±0.14^{b}	1.49±0.01^{b}	13.34±0.15^{b}	21.68±0.01^{b}	0.98±0.01^{b}	52.81±0.14^{d}	418.02±0.02^{b}
FR$_{50}$S$_{50}$	10.62±0.01^{a}	1.89±0.03^{a}	15.42±0.01^{a}	24.39±0.04^{a}	1.25±0.01^{a}	46.43±0.04^{e}	422.06±0.20^{a}
SR$_{90}$S$_{10}$	10.72±0.01^{c}	1.08±0.01^{d}	4.51±0.14^{e}	11.60±0.14^{e}	0.71±0.01^{d}	71.38±0.02^{a}	372.51±0.04^{e}
SR$_{80}$S$_{20}$	10.57±0.02^{c}	1.19±0.14^{c}	6.73±0.01^{d}	14.85±0.14^{d}	0.79±0.02^{c}	65.87±0.01^{b}	383.45±0.12^{d}
SR$_{70}$S$_{30}$	10.94±0.01^{b}	1.47±0.01^{b}	8.80±0.14^{c}	19.17±0.01^{c}	0.97±0.07^{b}	58.65±0.01^{c}	390.48±0.01^{c}
SR$_{60}$S$_{40}$	11.70±0.14^{a}	1.96±0.01^{a}	10.45±0.15^{b}	20.55±0.01^{b}	1.29±0.01^{a}	54.04±0.01^{d}	392.41±0.02^{b}
SR$_{50}$S$_{50}$	11.66±0.01^{a}	1.99±0.03^{a}	11.52±0.01^{a}	25.35±0.04^{a}	1.31±0.01^{a}	48.17±0.01^{e}	397.76±0.14^{a}
R$_{100}$	8.67±0.01	0.46±0.04	1.56±0.14	3.85±0.01	0.30±0.01	85.16±0.04	370.08±0.01

Values are expressed as mean ± Standard Deviation. Values with different superscripts on the same column are statistically different at $P<0.05$.
FRS = Fermented Rice/Sesame, SRS = Sprouted Rice/Sesame, R$_{100}$ = Rice, Subscripts = percentage substitution.

4.1.2 Proximate composition of extrudates

The mean observed values for proximate composition of the extrudates is presented in table 4.2. The moisture content ranged between 4.01 to 6.56 % and 5.51 to 7.95 % for extrudates produced from fermented and sprouted seed blends respectively. The results show significant ($P < 0.05$) increase in ash content with increase in sesame addition in the extrudates where the highest values were recorded in $FR_{50}S_{50}$ (2.01 %) and $SR_{50}S_{50}$ (2.46 %), while the least values were seen in $FR_{90}S_{10}$ (0.65 %) and $SR_{90}S_{10}$ (1.16 %) for the fermented and sprouted seed blends respectively. The protein content shows significant increase with sesame addition with highest values seen in $FR_{50}S_{50}$ (17.46 %) and $SR_{50}S_{50}$ (20.35 %), while the least values were seen in $FR_{90}S_{10}$ (7.26 %) and $SR_{90}S_{10}$ (8.28 %) from blends of fermented and sprouted seeds respectively. The fat content ranged between 2.05 % and 6.3 % for the extrudates produced from fermented seed blends with the highest value observed in $FR_{50}S_{50}$ (6.3 %). Lower fat contents (1.06 to 4.60 %) were recorded in extrudates produced from blends of sprouted seeds with the highest observed in $SR_{50}S_{50}$ (4.60 %) and the lowest in $SR_{90}S_{10}$ (1.06 %). The fibre contents varied between 0.43 to 1.33 % in the extrudates produced from blends of fermented rice and sesame flours, with the least value recorded in sample produced from $FR_{90}S_{10}$ (0.43 %) and the highest value observed in $FR_{50}S_{50}$ (1.33 %). Extrudates from the blends of sprouted seeds showed statistically higher fibre contents ranging between 0.77 to 1.62 % with the least and highest values recorded in $SR_{90}S_{10}$ (0.77 %) and $SR_{50}S_{50}$ (1.62 %) respectively. With respect to carbohydrate and the energy values, statistically ($P < 0.05$) higher values (93.79 % and 393.19 Kcal/100 g) were recorded in the control (R_{100}).

Table 4.2: Proximate composition of Extrudates

Sample	**Moisture (%)**	**Ash (%)**	**Fat (%)**	**Protein (%)**	**Fibre (%)**	**CHO (%)**	**Energy Value (Kcal)**
$FR_{90}S_{10}$	4.01±0.05^{e}	0.65±0.04^{c}	2.04±0.18^{e}	7.26±0.26^{e}	0.43±0.02^{d}	85.61±0.04^{a}	389.84±0.58^{d}
$FR_{80}S_{20}$	4.99±0.01^{c}	1.50±0.01^{b}	3.05±0.07^{d}	10.42±0.12^{d}	0.99±0.13^{b}	79.05±0.13^{b}	385.33±0.26^{e}
$FR_{70}S_{30}$	5.14±0.03^{b}	1.48±0.01^{b}	3.62±0.06^{c}	12.52±0.25^{c}	0.98±0.01^{b}	76.26±0.23^{c}	387.70±0.14^{c}
$FR_{60}S_{40}$	4.49±0.03^{d}	1.56±0.06^{b}	4.55±0.63^{b}	15.75±0.18^{b}	1.03±0.03^{b}	72.62±0.91^{d}	394.43±0.29^{a}
$FR_{50}S_{50}$	6.56±0.08^{a}	2.01±0.02^{a}	6.30±0.85^{a}	17.46±0.06^{a}	1.33±0.02^{a}	66.34±0.75^{e}	391.90±0.23^{b}
$SR_{90}S_{10}$	7.95±0.07^{a}	1.16±0.06^{e}	1.06±0.19^{e}	8.28±0.04^{e}	0.77±0.04^{e}	80.78±0.29^{a}	370.78±1.26^{c}
$SR_{80}S_{20}$	7.91±0.11^{a}	1.36±0.19^{d}	1.80±0.00^{d}	12.92±0.31^{d}	0.90±0.01^{d}	75.11±0.23^{b}	368.32±1.01^{d}
$SR_{70}S_{30}$	5.60±0.14^{c}	1.51±0.01^{c}	2.25±0.07^{c}	16.62±0.12^{c}	1.00±0.01^{c}	73.02±0.06^{c}	378.81±0.07^{b}
$SR_{60}S_{40}$	5.51±0.01^{c}	2.02±0.04^{b}	3.40±0.14^{b}	17.39±0.34^{b}	1.33±0.04^{b}	70.35±0.07^{d}	381.56±0.00^{a}
$SR_{50}S_{50}$	7.43±0.01^{b}	2.46±0.03^{a}	4.60±0.56^{a}	20.35±0.92^{a}	1.62±0.01^{a}	63.54±1.16^{e}	376.96±0.09^{b}
R_{100}	2.06±0.35	0.53±0.01	0.99±0.00	2.28±0.00	0.35±0.01	93.79±0.36	393.19±1.47

Values are expressed as mean ± Standard Deviation. Values with different superscripts on the same column are statistically different at $P < 0.05$.
FRS = Fermented Rice/Sesame, SRS = Sprouted Rice/Sesame, R_{100} = Rice, Subscripts = percentage substitution.

4.1.3 Selected vitamin B content of extrudates

The vitamins differed in composition and stability in all the extrudates as shown in table 4.3. The means scores recorded for vitamin B_1 (thiamine) in the extrudates ranged from 0.88 to 0.93 mg/100 g and 0.86 to 0.91 mg/100 g with increased sesame addition in the fermented and sprouted seed blends respectively. Vitamin B_2 (riboflavin) content of the extrudates from fermented and sprouted seed blends ranged from 0.14 to 0.47 mg/100 g and 0.40 to 0.79 mg/100 g with the least values (0.14 and 0.40 mg/100 g) observed in extrudates produced from $FR_{90}S_{10}$ and $SR_{90}S_{10}$, while the highest values (0.47 and 0.79 mg/100 g) were recorded in $FR_{50}S_{50}$ and $SR_{50}S_{50}$ respectively. Vitamin B_6 (Pyridoxine) was statistically higher in $FR_{50}S_{50}$ (0.27 mg/100 g) and $SR_{50}S_{50}$ (0.66 mg/100 g), and lower in $FR_{90}S_{10}$ (0.08 mg/100 g) and $SR_{90}S_{10}$ (0.30 mg/100 g) for the extrudates from fermented and sprouted seed blends with increased sesame addition respectively. The mean scores recorded for vitamin B_{12} (cobalamin) were statistically higher in $FR_{50}S_{50}$ (0.25 mg/100 g) and $SR_{50}S_{50}$ (0.58 mg/100 g) for the fermented and sprouted seed blends respectively. The least values of vitamin B_{12} were recorded in $FR_{90}S_{10}$ (0.34 mg/100 g) for the fermented seed blends while $SR_{90}S_{10}$, $SR_{80}S_{20}$, $SR_{70}S_{30}$ and $SR_{60}S_{40}$ showed no statistical difference ($P < 0.05$).

Table 4.3: Selected Vitamin B Content of Extrudates

Sample	Vit B_1 (mg/100 g)	Vit B_2 (mg/100 g)	Vit B_6 (mg/100 g)	Vit B_{12} (mg/100 g)
$FR_{90}S_{10}$	0.88 ± 0.14^a	0.14 ± 0.14^e	0.08 ± 0.14^c	0.34 ± 0.12^d
$FR_{80}S_{20}$	0.89 ± 0.14^a	0.27 ± 0.14^d	0.10 ± 0.14^c	0.43 ± 0.23^c
$FR_{70}S_{30}$	0.91 ± 0.14^a	0.35 ± 0.14^c	0.18±0.14b	0.65 ± 0.00^b
$FR_{60}S_{40}$	0.92 ± 0.14^a	0.40 ± 0.14^b	0.21 ± 0.10^b	0.68 ± 0.23^b
$FR_{50}S_{50}$	0.93 ± 0.14^a	0.47 ± 0.21^a	0.27 ± 0.14^a	1.11 ± 0.24^a
$SR_{90}S_{10}$	0.91 ± 0.28^a	0.40 ± 0.21^d	0.30 ± 0.07^d	0.25 ± 0.12^b
$SR_{80}S_{20}$	0.87 ± 0.14^a	0.42 ± 0.21^d	0.36 ± 0.14^c	0.25 ± 0.12^b
$SR_{70}S_{30}$	0.88 ± 0.14^a	0.49 ± 0.14^c	0.41 ± 0.21^b	0.28 ± 0.71^b
$SR_{60}S_{40}$	0.86 ± 0.42^a	0.61 ± 0.07^b	0.45 ± 0.14^b	0.30 ± 0.94^b
$SR_{50}S_{50}$	0.88 ± 0.07^a	0.79 ± 0.21^a	0.66 ± 0.14^a	0.58 ± 0.24^a
R_{100}	0.37±0.10	0.08±0.12	0.05±0.02	0.17±0.11

Values are expressed as mean± Standard Deviation. Values with different superscripts on the same column are statistically different at $P < 0.05$. FRS = Fermented Rice/Sesame, SRS = Sprouted Rice/Sesame, R_{100} = Rice, Subscripts = percentage substitution.

4.1.4 Mineral composition of extrudates

The mineral composition of the extrudates from fermented and sprouted seed blends of rice and sesame is presented in table 4.4. Phosphorus (P) was the most abundant mineral present in all the extrudates with increase in sesame addition and was statically higher in $FR_{60}S_{40}$ (2.30 g/100 g) and $FR_{50}S_{50}$ (2.26 g/100 g) for the fermented seed blends while it was higher in $SR_{50}S_{50}$ (3.42 g/100 g) for the sprouted seed blends. Statistically lower values of phosphorus were recorded in $FR_{90}S_{10}$ (1.20 g/100 g) and $SR_{90}S_{10}$ (1.90 g/100 g) for the fermented and sprouted seed blends respectively. Similarly, the results of sodium content in the fermented and sprouted seed blends were statically higher in $FR_{50}S_{50}$ (0.67 g/100 g) and $SR_{50}S_{50}$ (1.09 g/100 g), while the lower values were recorded in $FR_{90}S_{10}$ (0.49 g/100 g) to and $SR_{90}S_{10}$ (0.58 g/100 g) for the fermented and sprouted seed blends respectively. The results of potassium was statistically ($P < 0.05$) higher in $FR_{50}S_{50}$ (0.74 g/100 g) and $SR_{50}S_{50}$ (0.86 g/100 g), while the lower values were recorded in $FR_{90}S_{10}$ (0.31 g/100 g) and $SR_{90}S_{10}$ (0.51 g/100 g) in extrudates produced from the fermented and sprouted seed blends respectively. The calcium content increased significantly ($P < 0.05$) with increase in sesame addition and was higher in the blends of $FR_{50}S_{50}$ (0.68 g/100 g) and $SR_{50}S_{50}$ (1.58 g/100 g) and lower in the blends of $FR_{90}S_{10}$ (0.13 g/100 g) and $SR_{90}S_{10}$ (0.53 g/100 g) for extrudates produced from fermented and sprouted seed blends respectively. The findings in this work indicated statistically higher ($P < 0.05$) magnesium contents in $FR_{50}S_{50}$ (0.58 g/100 g) and $SR_{50}S_{50}$ (2.06 mg/100 g), and lower magnesium content in $FR_{90}S_{10}$ (0.29 g/100 g) and $SR_{90}S_{10}$ (0.37 g/100 g) for the extrudates from fermented and sprouted seed blends respectively. With regard to the iron content, statistically higher values ($P < 0.05$) were recorded in $FR_{50}S_{50}$ (9.42 mg/100 g) and $SR_{50}S_{50}$ (9.07 mg/100 g) while lower values were observed in $FR_{90}S_{10}$ (2.83 mg/100 g) and $SR_{90}S_{10}$ (3.51 mg/100 g) for extrudates from the fermented and sprouted seed extrudates respectively. The zinc content was found to be statistically higher ($P < 0.05$) in $FR_{60}S_{40}$ (0.94 mg/100 g) and $SR_{60}S_{40}$ (1.71 mg/100 g), and lower in $FR_{90}S_{10}$ (0.38 mg/100 g) and $SR_{90}S_{10}$ (0.66 mg/100 g) for the fermented and sprouted seed extrudates respectively.

Sample	Na (g/100g)	K (g/100g)	Ca (g/100g)	Mg (g/100g)	P (g/100g)	Fe (mg/100g)	Zn (mg/100g)
FR$_{90}$S$_{10}$	0.49±0.01^{cd}	0.31±0.04^{d}	0.13±0.01^{e}	0.29±0.74^{c}	1.20±0.85^{e}	2.83±0.01^{e}	0.38±0.01^{e}
FR$_{80}$S$_{20}$	0.48±0.01^{d}	0.50±0.03^{c}	0.37±0.04^{d}	0.33±0.01^{c}	1.28±1.43^{d}	4.12±0.01^{d}	0.56±0.01^{d}
FR$_{70}$S$_{30}$	0.50±0.02^{c}	0.62±0.01^{b}	0.50±0.03^{c}	0.51±0.02^{b}	1.70±0.86^{c}	7.21±0.01^{c}	0.80±0.00^{b}
FR$_{60}$S$_{40}$	0.53±0.03^{b}	0.65±0.00^{b}	0.56±0.02^{b}	0.51±0.02^{b}	2.30±1.56^{a}	7.89±0.13^{b}	0.94±0.01^{a}
FR$_{50}$S$_{50}$	0.67±0.02^{a}	0.74±0.03^{a}	0.68±0.03^{a}	0.58±0.03^{a}	2.26±2.19^{a}	9.42±0.06^{a}	0.67±0.01^{c}
SR$_{90}$S$_{10}$	0.58±0.04^{c}	0.51±0.01^{d}	0.53±0.03^{e}	0.37±0.13^{e}	1.90±1.65^{e}	3.51±0.01^{e}	0.66±0.01^{e}
SR$_{80}$S$_{20}$	0.53±0.03^{c}	0.65±0.01^{c}	0.66±0.06^{d}	0.45±0.01^{d}	2.03±0.85^{d}	3.64±0.03^{d}	0.73±0.01^{d}
SR$_{70}$S$_{30}$	0.62±0.01^{b}	0.73±0.01^{b}	0.74±0.04^{c}	1.05±0.06^{c}	2.36±0.07^{c}	4.14±0.04^{c}	0.81±0.01^{c}
SR$_{60}$S$_{40}$	0.63±0.03^{b}	0.84±0.03^{a}	0.99±0.08^{b}	1.35±0.05^{b}	2.95±0.14^{b}	5.68±0.04^{b}	1.71±0.02^{a}
SR$_{50}$S$_{50}$	1.09±0.02^{a}	0.86±0.00^{a}	1.58±0.05^{a}	2.06±0.04^{a}	3.42±0.00^{a}	9.07±0.01^{a}	1.20±0.01^{b}
R$_{100}$	0.15±0.01	0.17±0.00	0.09±0.01	0.19±0.01	0.54±0.00	1.83±0.03	0.11±0.01

Values are expressed as mean ± Standard Deviation. Values with different superscripts on the same column are statistically different at $P < 0.05$. FRS=Fermented Rice/Sesame, SRS = Sprouted Rice/Sesame, R$_{100}$ = Rice, Subscripts = percentage substitution.

4.1.5 Essential amino acid composition of extrudates

The essential amino acid composition of fermented and sprouted rice-sesame extrudates is presented in table 4.5. Leucine was found to be the most predominant amino acid in all the extrudates with statistically ($P < 0.05$) highest values recorded in $FR_{80}S_{20}$ (8.00 g/100 g protein) and $SR_{50}S_{50}$ (9.15 g/100g protein), and the least recorded in $FR_{50}S_{50}$ (6.37 g/100 g protein) and $SR_{70}S_{30}$ (7.08 g/100 g protein) for the fermented and sprouted seed blends respectively. For the isoleucine content of the extrudates from the fermented and sprouted seed blends, statistically higher values were recorded in $FR_{90}S_{10}$ (4.52 g/100 g protein) and $SR_{90}S_{10}$ (4.95 g/100 g protein) while the lowest values were observed in $FR_{50}S_{50}$ (3.77 g/100 g protein) and $SR_{80}S_{20}$ (3.10 g/100 g protein) respectively. The lysine content of the extrudates were statistically higher in $FR_{60}S_{40}$ (5.59 g/100 g protein) and $SR_{50}S_{50}$ (5.23 g/100 g protein) with the lowest values recorded in $FR_{90}S_{10}$ (3.74 g/100 g protein) and $SR_{80}S_{20}$ (4.03 g/100 g protein) for fermented and sprouted seed blends respectively. With regards to methionine content of the extrudates, statistically higher values were recorded in $FR_{80}S_{20}$ (2.92 g/100 g protein) and $SR_{80}S_{20}$ (3.45 g/100 g protein) while the lowest values were recorded in $FR_{70}S_{30}$ and $FR_{60}S_{40}$ (2.10 g/100 g protein) for the fermented seed blends and (2.18 g/100 g protein) for the sprouted seed blends respectively. It is also clear from the results in Table 6 that the statistically higher valine contents were recorded in $FR_{70}S_{30}$ (4.73 g/100 g protein) and $SR_{60}S_{40}$ (4.05 g/100 g protein) while the lower values were recorded in $FR_{60}S_{40}$ (2.09 g/100 g protein) and $SR_{80}S_{20}$ (2.70 g/100 g protein) for the fermented and sprouted seed blends respectively. Similarly, histidine content of extrudates produced from the blends of the fermented seeds varied from 0.89 to 1.70 g/100 g protein with the least value (0.89 g/100 g protein) recorded in $FR_{90}S_{10}$ and highest value (1.70 g/100 g protein) in $FR_{60}S_{40}$. In the sprouted seed extrudates, a range of 0.74 to 1.52 g/100 g protein was observed with the least value (0.74 g/100 g protein) recorded in $SR_{90}S_{10}$ while the highest value (1.52 g/100 g protein) was recorded in $SR_{70}S_{30}$. With regard to phenylalanine content of the extrudates, statistically higher values were recorded in $FR_{10}S_{10}$ (3.71 g/100 g protein) and $SR_{90}S_{10}$ (3.95 g/100 g protein) and lower in $FR_{50}S_{50}$ (2.69 g/100 g protein) for the fermented seed blends whereas there was no significant difference in $SR_{60}S_{40}$ (3.14 g/100 g protein) and $SR_{50}S_{50}$ (3.13 g/100 g protein) for the sprouted seed blends. The Arginine content increased with sesame addition in the formulations and was statistically higher in $FR_{50}S_{50}$ (5.16 g/100 g protein) and $SR_{50}S_{50}$ (4.58 g/100 g protein) and lower in $FR_{90}S_{10}$ (3.51 g/100 g protein) and $SR_{90}S_{10}$ (3.73 g/100 g protein) for the fermented and sprouted seed extrudates respectively. The threonine content was statistically higher in $FR_{60}S_{40}$ (4.70 g/100 g protein) and $SR_{60}S_{40}$ (4.92 g/100 g protein) and lower in $FR_{70}S_{30}$ (4.11 g/100 g protein) and $SR_{90}S_{10}$ (3.71 g/100 g protein) for the fermented and sprouted seed extrudates respectively. For the tryptophan content of the extrudates, statistically higher values were recorded in $FR_{50}S_{50}$ (2.96 g/100 g protein) and $SR_{60}S_{40}$ (2.66 g/100 g protein) and lower in $FR_{90}S_{10}$ (2.51 g/100 g protein) and $SR_{80}S_{20}$ (2.13 g/100 g protein) for the fermented and sprouted seed blends respectively.

Table 4.5: Essential Amino Acid Composition of Extrudates

Samples	**Leucine (mg/100g)**	**Isoleucine (mg/100g)**	**Lysine (mg/100g)**	**Methionine (mg/100g)**	**Valine (mg/100g)**	**Histidine (mg/100g)**	**Phenylalanine (mg/100g)**	**Arginine (mg/100g)**	**Threonine (mg/100g)**	**Tryptophan (mg/100g)**
$FR_{90}S_{10}$	7.42 ± 0.38^{c}	4.52 ± 0.70^{a}	3.74 ± 0.44^{d}	2.49 ± 0.28^{b}	4.19 ± 0.40^{b}	0.89 ± 0.19^{d}	3.71 ± 0.52^{a}	3.51 ± 0.12^{e}	4.16 ± 0.05^{d}	2.51 ± 0.17^{b}
$FR_{80}S_{20}$	8.00 ± 0.19^{a}	4.11 ± 0.01^{b}	4.55 ± 0.67^{c}	2.92 ± 0.07^{a}	3.22 ± 0.10^{c}	1.02 ± 0.16^{c}	3.59 ± 0.44^{ab}	3.68 ± 0.36^{d}	4.47 ± 0.24^{b}	2.52 ± 0.13^{b}
$FR_{70}S_{30}$	6.95 ± 0.78^{d}	3.98 ± 0.08^{d}	5.03 ± 0.14^{b}	2.10 ± 0.01^{d}	4.73 ± 0.06^{a}	1.47 ± 0.20^{b}	3.15 ± 0.06^{bc}	4.08 ± 0.01^{c}	4.11 ± 0.03^{d}	2.52 ± 0.13^{b}
$FR_{60}S_{40}$	7.74 ± 0.53^{b}	4.07 ± 0.05^{c}	5.59 ± 0.64^{a}	2.10 ± 0.01^{d}	2.09 ± 0.02^{d}	1.70 ± 0.16^{a}	2.88 ± 0.21^{c}	4.70 ± 0.05^{b}	4.70 ± 0.16^{a}	2.92 ± 0.41^{a}
$FR_{50}S_{50}$	6.37 ± 0.35^{c}	3.77 ± 0.57^{e}	5.06 ± 0.62^{b}	2.20 ± 0.02^{c}	4.15 ± 0.06^{b}	1.41 ± 0.16^{b}	2.69 ± 0.12^{d}	5.16 ± 0.05^{a}	4.17 ± 0.07^{c}	2.96 ± 0.11^{a}
$SR_{90}S_{10}$	8.04 ± 0.24^{b}	4.95 ± 0.15^{a}	4.47 ± 0.64^{c}	2.71 ± 0.29^{b}	4.03 ± 0.04^{a}	0.74 ± 0.13^{d}	3.95 ± 0.34^{a}	3.73 ± 0.06^{d}	3.71 ± 0.16^{d}	2.55 ± 0.10^{b}
$SR_{80}S_{20}$	9.11 ± 0.64^{a}	3.10 ± 0.02^{e}	4.03 ± 0.06^{e}	3.45 ± 0.58^{a}	2.70 ± 0.56^{b}	1.43 ± 0.11^{b}	3.73 ± 0.06^{b}	3.95 ± 0.05^{c}	4.39 ± 0.32^{b}	2.13 ± 0.02^{d}
$SR_{70}S_{30}$	7.08 ± 0.38^{d}	4.57 ± 0.15^{b}	4.73 ± 0.33^{b}	2.74 ± 0.12^{b}	4.00 ± 0.02^{a}	1.52 ± 0.02^{a}	3.59 ± 0.41^{c}	3.91 ± 0.11^{c}	4.11 ± 0.23^{c}	2.24 ± 0.17^{cd}
$SR_{60}S_{40}$	7.40 ± 0.57^{c}	4.23 ± 0.02^{c}	4.37 ± 0.25^{d}	2.18 ± 0.01^{d}	4.05 ± 0.05^{a}	1.30 ± 0.12^{c}	3.14 ± 0.04^{d}	4.29 ± 0.32^{b}	4.92 ± 0.06^{a}	2.66 ± 0.01^{a}
$SR_{50}S_{50}$	9.15 ± 1.16^{a}	4.09 ± 0.02^{d}	5.23 ± 0.19^{a}	2.48 ± 0.1^{c}	3.49 ± 0.60^{ab}	1.46 ± 0.20^{b}	3.13 ± 0.02^{d}	4.58 ± 0.05^{a}	4.11 ± 0.01^{c}	2.30 ± 0.17^{c}
R_{100}	2.93±0.14	1.52±0.07	0.66±0.13	0.72±0.03	1.78±0.09	0.67±0.02	2.30±0.05	2.26±0.08	2.17±0.11	0.93±0.01

Values are expressed as mean± Standard Deviation. Values with different superscripts on the same column are statistically different at $P < 0.05$. FRS = Fermented Rice/Sesame, SRS = Sprouted Rice/Sesame, R_{100} = Rice, Subscripts = percentage substitution.

4.1.6 Antinutrients composition of extrudates

The antinutrienst composition of extrudates is presented in table 4.6. The tannin composition of the extruded samples ranged from 0.12 to 0.31 mg/mL with increase in sesame addition in the fermented seed blends while the range of 0.14 to 0.30 mg/mL was recorded for the sprouted seed blends. The mean scores for phytate content of the extrudates ranged from 0.98 to 1.16 mg/mL and 0.58 to 0.95 mg/mL for the fermented and sprouted seed blends respectively. The oxalate content of the extrudates was statistically higher ($P < 0.05$) in $FR_{50}S_{50}$ (2.28 mg/mL) and $SR_{50}S_{50}$ (3.54 mg/mL) and lower in $FR_{90}S_{10}$ (0.17 mg/mL) and $SR_{90}S_{10}$ (2.68 mg/mL) for the fermented and sprouted seed blends respectively.

Table 4.6: Antinutrients Composition of Extrudates

Sample	Tannin (mg/mL)	Phytate (mg/mL)	Oxalate (mg/mL)
$FR_{90}S_{10}$	0.12 ± 0.02^{c}	0.98 ± 0.20^{c}	0.17 ± 0.06^{d}
$FR_{80}S_{20}$	0.23 ± 0.03^{b}	1.03 ± 0.01^{b}	1.11 ± 0.02^{c}
$FR_{70}S_{30}$	0.28 ± 0.02^{ab}	1.05 ± 0.01^{b}	1.12 ± 0.03^{c}
$FR_{60}S_{40}$	0.30 ± 0.02^{a}	1.16 ± 0.01^{a}	1.96 ± 0.10^{b}
$FR_{50}S_{50}$	0.31 ± 0.01^{a}	1.16 ± 0.00^{a}	2.28 ± 0.00^{a}
$SR_{90}S_{10}$	0.30 ± 0.02^{a}	0.58 ± 0.27^{d}	2.68 ± 0.06^{d}
$SR_{80}S_{20}$	0.26 ± 0.01^{ab}	0.84 ± 0.01^{c}	2.99 ± 0.06^{c}
$SR_{70}S_{30}$	0.24 ± 0.04^{b}	0.85 ± 0.00^{c}	3.23 ± 0.10^{b}
$SR_{60}S_{40}$	$0.19.\pm0.01^{b}$	0.92 ± 0.01^{b}	3.27 ± 0.03^{b}
$SR_{50}S_{50}$	0.13 ± 0.01^{c}	0.95 ± 0.01^{a}	3.54 ± 0.03^{a}
R_{100}	0.11±0.01	0.53±0.02	0.04±0.02

Values are expressed as mean ± Standard Deviation. Values with different superscripts on the same column are statistically different at $P < 0.05$. FRS = Fermented Rice/Sesame, SRS = Sprouted Rice/Sesame, R_{100} = Rice, Subscripts = percentage substitution.

4.1.7 Functional properties of extrudates

The functional properties of extrudates presented in table 4.7 show significant variability in the bulk density, water absorption capacity, water solubility index, swelling capacity and swelling power. The bulk density of extrudates from fermented and sprouted seed blends was statistically higher ($P < 0.05$) in $FR_{90}S_{10}$ and $FR_{80}S_{20}$ (0.58 g/cm^3) and $SR_{90}S_{10}$ (0.57 g/cm^3) and lower in $FR_{50}S_{50}$ (0.54 g/cm^3) and $SR_{50}S_{50}$ (0.49 g/cm^3) respectively. The bulk density (BD) of extrudates decreased significantly ($P < 0.05$) with the incorporation of sesame compared to the control (3.10 g/cm^3). The water absorption capacity (WAC) of the different formulations ranged between 3.52 to 4.80 g/g and 3.50 to 4.74 g/g in blends of fermented and sprouted seed extrudates with the the higher values recorded in $FR_{50}S_{50}$ (4.80 g/g) and $SR_{50}S_{50}$ (4.74 g/g) and lower values recorded in $FR_{90}S_{10}$ (3.52 g/g) and $SR_{90}S_{10}$ (3.50 g/g) respectively. The mean scores recorded for swelling capacity (SC) of the fermented and sprouted seed extrudates were statistically higher in $FR_{90}S_{10}$ (4.26 g/g) and $SR_{90}S_{10}$ (5.99 g/g), and lower in $FR_{50}S_{50}$ (2.97 g/g) and $SR_{50}S_{50}$ (4.30 g/g) respectively. With regard to the swelling power (SP), statistically higher values were recorded in $FR_{90}S_{10}$ (2.91 %) and $SR_{90}S_{10}$ (5.37 %) and lower in $FR_{50}S_{50}$ (1.89 %) and SRS (1.08 %) for the fermented and sprouted seed extrudates respectively. There was significant increase in the SP values when compared to that of the control (2.00 %) except in the extrudates produced from equal proportions ($FR_{50}S_{50}$) of fermented rice and sesame flours (0.89 %). The mean scores recorded for the water solubility index (WSI) was significantly higher in the R_{100} (7.98 %) and lower in $FR_{50}S_{50}$ (5.35 %) and $SR_{50}S_{50}$ (5.00 %) for the fermented and sprouted seed extrudates.

Table 4.7: Functional Properties of Extrudates

Sample	Bulk density (g/cm^3)	Water Absorption (g/g)	Swelling Capacity (%)	Swelling Power (%)	Water Solubility Index (%)
$FR_{90}S_{10}$	$0.58±0.10^a$	$3.52±0.71^e$	$4.26±1.41^a$	$2.91±0.71^a$	$7.65±0.49^a$
$FR_{80}S_{20}$	$0.58±0.36^a$	$4.22±0.91^d$	$3.32±2.12^b$	$2.80±0.71^b$	$5.95±0.35^b$
$FR_{70}S_{30}$	$0.57±0.14^b$	$4.40±1.41^c$	$3.21±0.71^c$	$2.73±1.41^c$	$5.70±0.42^c$
$FR_{60}S_{40}$	$0.56±0.42^c$	$4.51±0.71^b$	$3.11±1.41^d$	$2.72±0.21^c$	$5.62±0.35^d$
$FR_{50}S_{50}$	$0.54±0.23^d$	$4.80±2.72^a$	$2.97±0.71^e$	$1.89±0.01^d$	$5.35±0.04^e$

SR$_{90}$S$_{10}$	0.57 ± 0.13^{a}	3.50 ± 0.00^{d}	5.99 ± 0.92^{a}	5.37 ± 0.22^{a}	6.85 ± 0.21^{a}
SR$_{80}$S$_{20}$	0.53 ± 0.71^{ab}	4.16 ± 1.48^{c}	5.95 ± 0.42^{b}	5.02 ± 0.09^{b}	5.90 ± 0.14^{b}
SR$_{70}$S$_{30}$	0.51 ± 0.10^{b}	4.18±0.85c	5.49 ± 0.28^{c}	3.47 ± 0.71^{c}	5.40 ± 0.14^{c}
SR$_{60}$S$_{40}$	0.51 ± 0.10^{b}	4.68 ± 1.41^{b}	4.90 ± 0.71^{d}	1.52 ± 0.36^{d}	5.40 ± 0.14^{c}
SR$_{50}$S$_{50}$	0.49 ± 0.04^{c}	4.74 ± 0.64^{a}	4.30 ± 1.06^{e}	1.06 ± 0.36^{e}	5.00 ± 0.00^{d}
R$_{100}$	3.10±0.19	2.32±0.14	4.77±0.76	2.00±0.01	7.98±0.01

Values are expressed as mean ± Standard Deviation. Values with different superscripts on the same column are statistically different at $P < 0.05$. FRS = Fermented Rice/Sesame, SRS = Sprouted Rice/Sesame, R$_{100}$ = Rice, Subscripts = percentage substitution.

4.1.8 Sensory evaluation of extrudates

Mean scores of sensory attributes of extrudates evaluated are presented in table 4.8. The mean scores for appearance was statistically ($P < 0.05$) higher in the R$_{100}$ (7.35 ± 0.11) than the highest scores observed in FR$_{90}$S$_{10}$ (6.90 ± 1.48) and SR$_{90}$S$_{10}$ (6.85 ± 0.81) for the fermented and sprouted seed extrudates respectively. The taste for the extruded blends was statistically higher in FR$_{50}$S$_{50}$ (6.85 ± 0.67) and SR$_{50}$S$_{50}$ (6.55 ± 0.95) and lower in FR$_{90}$S$_{10}$ (5.45 ± 0.98) and SR$_{70}$S$_{30}$ (5.10 ± 0.64) for the fermented and sprouted seeds respectively. With regards to the aroma, statistically ($P < 0.05$) higher values were recorded in FR$_{60}$S$_{40}$ (6.95 ± 1.07) for the fermented seed extrudates, while SR$_{60}$S$_{40}$ (7.15 ± 1.22) and SR$_{50}$S$_{50}$ (7.15 ± 1.25) were not statistically different at ($P < 0.05$) for the sprouted seed blends. The lower values for the aroma were recorded in FR$_{90}$S$_{10}$ (5.15 ± 1.03) and SR$_{90}$S$_{10}$ (6.00 ± 1.12) for the fermented and sprouted seed blends respectively. The mouthfeel of the extrudates from the fermented and sprouted seed blends increased with sesame addition and the higher values were recorded in FR$_{50}$S$_{50}$ (6.25 ± 0.91) and SR$_{50}$S$_{50}$ (6.30 ± 0.65), wwhile the least values were recorded in FR$_{90}$S$_{10}$ (5.30 ± 0.92) and SR$_{90}$S$_{10}$ (5.20 ± 1.19) respectively. The mean scores for the aftertaste of the extrudates was statistically higher in R$_{100}$ (6.53 ± 0.01) than FR$_{90}$S$_{10}$ (5.45) and FR$_{80}$S$_{20}$ (5.49 ± 0.88) for the fermented seed blends, and SR$_{90}$S$_{10}$ (5.90 ± 1.07) for the sprouted seed blends. Furthermore, the general acceptability of extruded blends was significantly higher in $FR_{60}S_{40}$ (6.95 ± 1.09) and $SR_{60}S_{40}$ (6.60 ± 0.50) for the fermented and sprouted seed blends respectively.

Table 4.8: Sensory Evaluation of Extrudates

Sample	Appearance	Taste	Aroma	Mouthfeel	Aftertaste	Overall Acceptability
$\mathbf{FR_{90}S_{10}}$	$6.90{\pm}1.48^a$	$5.45{\pm}0.98^e$	$5.15{\pm}1.03^d$	$5.30{\pm}0.92^c$	$5.45{\pm}0.83^a$	$5.15{\pm}0.51^e$
$\mathbf{FR_{80}S_{20}}$	$6.80{\pm}0.95^b$	$5.65{\pm}1.22^d$	$5.95{\pm}1.09^c$	$6.15{\pm}0.58^b$	$5.49{\pm}0.88^a$	$5.70{\pm}0.86^d$
$\mathbf{FR_{70}S_{30}}$	$6.80\ {\pm}0.83^b$	$6.20{\pm}0.76^b$	$5.98{\pm}0.86^c$	$6.15{\pm}0.93^b$	$5.40{\pm}0.50^b$	$6.20{\pm}0.62^c$
$\mathbf{FR_{60}S_{40}}$	$6.00{\pm}0.80^d$	$6.10{\pm}0.85^c$	$6.82{\pm}1.13^b$	$6.20{\pm}0.72^{ab}$	$5.25{\pm}0.55^c$	$6.95\ {\pm}1.09^a$
$\mathbf{FR_{50}S_{50}}$	$6.60\ {\pm}1.14^c$	$6.85{\pm}0.67^a$	$6.95{\pm}1.07^a$	$6.25{\pm}0.91^a$	$5.05{\pm}0.68^d$	$6.85\ {\pm}1.05^b$
$\mathbf{SR_{90}S_{10}}$	$6.85{\pm}0.81^a$	$5.10{\pm}0.64^e$	$6.00{\pm}1.12^d$	$5.20{\pm}1.19^e$	$5.90{\pm}1.07^a$	$5.80{\pm}0.89^d$
$\mathbf{SR_{80}S_{20}}$	$6.50{\pm}0.76^c$	$5.56{\pm}0.69^c$	$6.15{\pm}0.49^c$	$5.45{\pm}0.60^d$	$5.55{\pm}0.49^b$	$5.65{\pm}0.58^e$
$\mathbf{SR_{70}S_{30}}$	$6.70{\pm}0.92^b$	$5.45{\pm}1.05^d$	$6.55{\pm}1.57^b$	$5.85{\pm}0.81^c$	$5.20{\pm}0.95^c$	$6.54{\pm}0.68^c$
$\mathbf{SR_{60}S_{40}}$	$5.80{\pm}0.61^c$	$6.20{\pm}0.69^b$	$7.15{\pm}1.22^a$	$6.20{\pm}0.95^b$	$5.21{\pm}0.51^c$	$6.60{\pm}0.50^a$
$\mathbf{SR_{50}S_{50}}$	$5.55{\pm}1.09^d$	$6.55{\pm}0.95^a$	$7.15{\pm}1.25^a$	$6.30{\pm}0.65^a$	$5.20{\pm}0.67^c$	$6.50{\pm}0.82^b$
$\mathbf{R_{100}}$	7.35±0.11	5.06±0.15	4.22±0.13	5.00±0.13	6.53±0.01	6.30±0.14

Values are expressed as mean ± Standard Deviation. Values with different superscripts on the same column are statistically different at $P < 0.05$. FRS = Fermented Rice/Sesame, SRS = Sprouted Rice/Sesame, R_{100} = Rice, Subscripts = percentage substitution.

4.2 Discussion of Results

4.2.1 Proximate composition of fermented and sprouted rice-sesame composites

The moisture content of $SR_{60}S_{40}$ (11.70 ± 0.14 %) and $SR_{50}S_{50}$ (11.66 ± 0.01 %) were highest across the formulated blends. High moisture content above 10 % has been associated with short shelf life as this encourages microbial proliferation that lead to spoilage (Danbaba *et al*., 2016)). The higher moisture contents observed in some of the blends might be due to handling of samples in the laboratory and some of moisture content in the zip-lock bags during packaging. The ash content which is the indication of mineral content increased with increased sesame addition in the formulated blends. This was in agreement with an earlier study by Akusu *et al*. (2019) when wheat flour was substituted with fermented sesame flour (13.40 – 14.98 %) and sprouted sesame flour (8.50 – 9.42 %) for the production of cookies. The significantly higher ($P < 0.05$) ash contents observed in the blends of the sprouted seed blends when compared to those of the fermented seed blends could be attributed to addition effect caused by germinated sesame flour since sesame flour contains higher amount of ash and crude fibre (El-Adawy, 2002). Fat is essential component of tissues and a veritable source for fat soluble vitamins (A, D, E and K). It is able to supply thrice the amount of energy required by the body (Wardlaw, 2004).

Danbaba *et al.* (2016) reported that for a food to be used as complementary formulations, the minimum fat content requirement should be 6 %. The observed decrease in the fat content of the sprouted seeds flours might be due to the increased activities of lipolytic enzymes during germination. These enzymes hydrolyze fats to simpler products which can be used as a source of energy for the developing embryo. The decreased fat content implies an increased shelf life for the germinated seeds flours compared to the fermented counterparts. The protein content in all the samples increased significantly ($P < 0.05$) with increase in percentage substitution of processed sesame seed flour. Composites produced from fermented and sprouted rice/sesame seed flour at substitution level of 50 % with 50 % sesame flour gave significantly higher crude protein of 24.39 % and 25.35 %, respectively. The slightly higher protein content observed in the composites produced from the sprouted seeds could be attributed to loss of dry weight as some carbohydrates and fats are utilized for respiration but also some amino acids are synthesized during germination (Jan *et al.*, 2017). Jimoh *et al.*, (2011) reported increase in protein content of defatted sesame seed from 21.78 % to 42.21 %. Similar increase in total protein content of cookies produced with soy-flour substitution in the range of 8.75 % to 24.65 % in wheat flour has been reported by earlier researchers (Ndife *et al.*, 2014). The observed increase therefore, could also be attributed to defatting of the processed sesame flours. Other studies have also reported a similar increase of protein content in composite flours (Singh *et al*., 2000; Mashayekh *et al*., 2008). Protein is a major nutrient needed as building blocks for the body, necessary for growth and for the repair of damaged tissues (Wardlaw, 2004). The observed crude fibre contents of the sprouted composites were significantly higher than that of their fermented counterparts with increased sesame addition. This could be attributed to addition effect caused by germinated sesame flour since sesame flour contains higher amount of crude fibre (El-Adawy, 2002). Carbohydrate was shown to reduce with increased substitution of processed sesame seed flour. Increased fibre and the lower carbohydrate content observed in both fermented and sprouted seed composited could have several health benefits, as it will aid digestion in the colon and reduce constipation often associated with products from refined grain flours (Elleuch *et al*., 2011).

4.2.2 Proximate composition of extrudates

The results obtained for the proximate composition (moisture, protein, lipid, fibre, ash, carbohydrate and caloric value) of fermented and sprouted rice-sesame extrudates shows the moisture contents were reduced to between 4.01 to 6.56 % and 5.51 to 7.95 % for extrudates produced from fermented and sprouted flours respectively. Several authors including Danbaba *et al*. (2016) stated that in a dry food system with moisture content between 6 % and 10 %, there is prolonged shelf stability, and above this range, the stability of the system could be impeded by both chemical and microbiological agents. These results therefore, suggested that the products had low moisture enough to have an extended shelf life. The ash content which is the indication of mineral content of the extrudates increased after extrusion. The results show significant increase in ash content with increase in sesame flour addition in the extrudates. These results are in agreement with an earlier observation by El-Samahy *et al*., (2007), who observed significant increase in ash content (0.31 – 0.54 %) when cactus pear concentration was increased in a rice-cactus pear extruded samples. The protein content of extrudates decreased upon extrusion of the blends. This trend was also observed by Anuonye *et al*. (2010). This decrease might be due to the gelatinization effect of the extrusion processing (Rampersad *et al*., 2003). Reduction in protein content led to increase in the calorific value. This was expected and was in conformity with reported trends (Anuonye *et al*., 2010; Iwe *et al*., 2001). Increase in sesame flour addition indicated proportional increase in protein content of the extrudates.

Filli *et al*. (2011) reported an increase from 11.23 to 16.23 % protein content when millet was fortified with cowpea through extrusion cooking. The slightly higher protein content observed in the samples produced from the sprouted seeds could be attributed to loss of dry weight as some carbohydrates and fats are utilized for respiration but also some amino acids are synthesized during germination (Jan *et al*., 2017). The lower fat contents (1.06 to 4.60 %) were recorded in extrudates produced from blends of sprouted rice and sesame blends. Rice has little oil as seen in the control (0.99 %) compared to sesame which is an oil seed. Other studies have reported that germination reduces fat content due to hydrolysis and utilization of fats as an energy source for biochemical reactions (Nkhata *et al*., 2018). However, the extruder processing condition further reduced the fat present in the blends through several reactions including complex formation with amylose and protein, oxidation, lipid binding due to interactions with starch and proteins, cis and trans isomerization of unsaturated fatty acids and degradation of fat splitting enzymes. All these reactions resulted in more fat loss when compared to their unextruded composites. Danbaba *et al*., (2016) reported that for a food to be used as complementary formulation, the minimum fat content requirement should be 6 %. Dietary fats are beneficial in the body because of their function as carriers of fat soluble vitamins in the diet and as mediators of some physiological processes associated with growth and development, inflammation and brain function (Gbenyi *et al*., 2016). The significantly low fat content implies that this quality parameter needs to be added from other sources into the diet of the consumers of this product especially if it is going to be used as weaning food. The fibre contents of the extrudates increased significantly after extrusion when compared to that of the blends. The relatively higher fibre content recorded in the extrudates of sprouted seed blends could be an indication of mineral bioavailability in the samples. It may also be attributed to addition effect caused by germinated sesame flour since sesame flour contains higher amount of ash and crude fibre (El-Adawy, 2002). The results of carbohydrate showed significant ($P < 0.05$) decrease in carbohydrate values with increase in sesame flours for both fermented and sprouted samples when compared to that of the control sample (93.79 % and 393.19 Kcal). This could be as a result of the decrease in rice content which is the major carbohydrate source in the formulations. Both fermentation and germination increases α-amylase activity and consequently increases the digestibility of starch, making them good methods in the preparation of complementary and infant foods (Nkhata *et al*., 2018).

4.2.3 Selected vitamin B content of extrudates

The vitamin contents differed in composition and stability in all the extrudates. There was no significant difference ($P < 0.05$) in the vitamin B_1 (thiamine) values within and between all the extrudates. A significant ($P < 0.05$) increase was observed in the thiamine retention of extrudates post extrusion cooking compared to that of the control

(0.37 mg/100 g). Thiamine values obtained for this study were higher than the RDI value (0.5 mg/100 g) for feeding infants below three years (Nestle, 2000). Thiamine helps the body maximize the use of carbohydrates, its major source of energy. It is also important for the proper functioning of the heart, nervous system, and muscle coordination (Eze *et al.*, 2020). Extrudates produced from the blends of fermented seed flours recorded significantly lower vitamin B_2 (riboflavin) contents compared to those produced from blends of the sprouted rice/sesame blends. Several authors including Nkhata *et al.*, (2018) reported that sprouting increases various vitamins present in cereals and legumes such as tocopherols (α-, β-, and γ- tocopherols), riboflavin, and total niacin due to synthesis of these vitamins by the new sprouts. The riboflavin contents of $SR_{60}S_{40}$ and $SR_{50}S_{50}$ met the RDA (0.5 mg/day) for children between 1 to 3 years, as recommended by the report of a joint FAO and WHO (2001) while the rest had significantly ($P < 0.05$) higher amounts of riboflavin when compared with R_{100} (0.05). Riboflavin is important for human nutrition and health (ICMR, 2002). With regard to the vitamin B_6 (Pyridoxine) contents of the extruded blends, significantly ($P < 0.05$) higher values were observed when compared to that of the fermented counterparts. This could also be attributed to the synthesis of pyridoxine by the new sprouts. The significantly ($P < 0.05$) higher values of vitamin B_{12} (cobalamin) recorded in extrudates of the fermented seed blends when compared to their corresponding sprouted seeds counterparts could be attributed to the fact that cobalamin is a water soluble vitamin and could have been lost during the longer steeping period of sprouting. Nkhata *et al.*, (2018) reported that losses of some water-soluble vitamins are common during germination due to leaching. The results from this study revealed that the extrudates would contribute substantially to the recommended dietary requirements of vitamins for both children and adults.

4.2.4 Mineral composition of extrudates

The mineral elements determined in the present study (Na, K, Ca, Mg, P, Fe and Zn) varied significantly ($P < 0.05$) among the extrudates. Phosphorus (P) was the most abundant mineral present in all the formulated blends. The values were significantly higher when compared to that of the extruded rice (R_{100} 0.54 g/100). Phytate has a high concentration in various food products and constitutes the key source of phosphorus storage in ripe grains and legumes. This may be attributed to increased hydrolysis of phytate during fermentation and sprouting. Phosphorus is of prime importance in the development of skeletal tissues, formation of nucleic acids (DNA and RNA), energy storage and transfer, structural roles (formation of cell membrane) and acid-base balance of cells (Iombor *et al*., 2016). The daily requirement of phosphorus (700 mg/day) for both men and women (pregnant and lactating) age 19 years and older (Food and Nutrition Board, 1997) could be easily provided by any of the rice-sesame extrudates. Sodium is one of the minerals whose high intake is considered as a factor in the etiology of hypertension; hence its low intake is encouraged (Kadan *et al*., 2003). The concentration of sodium observed in the control (R_{100}) was found to be 0.15 ± 0.01 g/100 g, and there was significant increase ($P < 0.05$) in sodium content in the developed extrudates, which could be linked to the presence of different concentrations of sesame flour. This agrees with the findings of Banki *et al*., (2021) that most cereals tend to have low sodium contents. The higher concentrations seen in the formulated blends could be due to the high antinutrient content of sesame whose breakdown during fermentation and sprouting greatly increases the mineral content of food. The highest concentration of sodium (1.09 g/100 g) was recorded in $SR_{50}S_{50}$. Thus, sodium is an essential cation required for acid–base balance, muscle contraction and regulation of osmotic pressure (Banki *et al*., 2021). Although, significantly higher scores were recorded in the extrudates from sprouted seed blends, there was significant increase in potassium contents of extrudates produced from both fermented and sprouted seeds flour blends with increase in sesame addition when compared to R_{100} (0.17 g/100 g). Potassium is integral part of phytate molecules where it is covalently bonded rendering it inaccessible by the digestive enzymes (Nkhata *et al*., 2018).

It has been hypothesized that the remarkable increase in phytase activity during sprouting helps reduce phytic acids, which bind minerals subsequently leading to increased mineral availability (Luo *et al*., 2014). However, Banki *et al*. (2021) attributed increase in potassium content when rice was extruded with pigeon pea to increased protease activity during extrusion cooking. Potassium is primarily an intracellular cation which is bound to protein and functions together with sodium in maintaining the normal pH (Banki *et al*., 2021). Its availability could serve as an added advantage for product development, especially complementary food where potassium is an important macro nutrient among other minerals. Calcium is an important bone related macro element in human nutrition. The calcium contents of all the extrudates were significantly ($P < 0.05$) higher than the value obtained for the control. Reddy *et al*. (2017) indicated that due to low calcium content of rice, blending with other pulses improves the functional properties and mineral compositions. Singh *et al*. (2000) attributed the increase in calcium, phosphorus and iron contents during the production of cereal-based products to feed blend composition and water content. Calcium is important in blood clotting, muscles contraction and in certain enzymes in metabolic processes (Akusu *et al*., 2020). Calcium plays an important role in prevention of rickets in children and Osteomalacia (the adult rickets) as well as osteoporosis (bone thinning) among older people (Banki *et al*., 2021). $SR_{50}S_{50}$ met the calcium recommended daily allowance (1000 mg) for lactating mothers while most of the extrudates could be estimated to fulfill more than 50

% of the required calcium recommended daily allowance for both men and women. The findings in this work indicated higher ($P < 0.05$) magnesium contents in the sprouted seeds extrudates (1.90 to 3.42 g/100 g) than the fermented seeds extrudates (0.29 to 0.58 g/100 g). In addition to the effect of fermentation and sprouting which influences mineral bioavailabilty in plant products, sesame addition significantly increased magnesium contents in the extrudates which could serve as an added advantage for product quality. However, Banki *et al*., (2021) attributed increased magnesium content during extrusion to the activities of protease, lipase and amylase during gelatinization of the extrudates. Magnesium as an activator of many enzyme systems aids in maintaining the electrical potential of the nerve cells. High amount of magnesium, calcium and potassium have been reported to reduce blood pressure in humans (Ufot *et al*., 2018).

Minerals like iron and zinc are often added to food for the improvement of nutritional composition. In this study, the results of both iron and zinc contents were observed to increase with sesame addition in both fermented and sprouted seed extrudates and were statistically higher ($P < 0.05$) than found in R_{100}. This increase could be attributed to destruction of antinutrients (oxalates and phytic acid) during fermentation, sprouting and extrusion of the extrudates. Adequate iron in the diet is essential to minimize the incidence of iron deficiency anemia, which is considered as the most common nutritional disorder worldwide (Ufot *et al*., 2018), while zinc is a component of living cells and essential for assisting enzyme reaction and wound healing (Agunbiade and Ojezele, 2010). Inadequate intakes of micronutrients (iron and zinc) have been associated with severe malnutrition, increased disease conditions and mental impairment (Akusu *et al*., 2020). The mineral content of all the extrudates were significantly higher than the control. The results from this study revealed that the extrudates would contribute substantially to the recommended dietary requirements for minerals.

4.2.5 Essential amino acid composition of extrudates

Leucine was found to be the most predominant essential amino acid in all the extruded blends. This agrees with the findings of Aremu *et al*. (2010) that plant products of Nigerian origin have leucine as the most abundant essential amino acid. The isoleucine content of the control (1.52 ± 0.07 g/100 g protein) was significantly lower than that of the fermented and sprouted rice/sesame extrudates. The limiting essential amino acids in rice and sesame are lysine and methionine respectively. Lysine is however thermolabile and thus; focusing on lysine retention during the extrusion process is of particular importance. The lysine content of the extruded blends was significantly higher ($P < 0.05$) than the 340 mg/gN recommended by WHO as recommended dietary allowance (RDA). Masatcioglu *et al*. (2014) found that decrease in lysine contents in some extruded products could be attributed to low moisture contents, longer period of extrusion and the nature of the extruder. With regards to methionine, significantly higher ($P < 0.05$) methionine contents were recorded in the sprouted seed extrudates when compared to the corresponding fermented seed extrudates. Scholars have reported a reduction in total proteins albeit with increase in specific amino acids such as lysine, tryptophan, and methionine after sprouting (Nkhata *et al*., 2018). The methionine content of extruded blends was statistically higher ($P < 0.05$) when compared to that of control (0.72 ± 0.03 g/100 g protein). Thus, $FR_{60}S_{40}$ and $SR_{80}S_{20}$ were statistically higher ($P < 0.05$) in limiting amino acids lysine (5.59 %) and methionine (3.45 %) respectively. These values were significantly higher ($P < 0.05$) than the values reported by Banki *et al*., (2021) for extruded rice-pigeon pea flour composites. It is also clear from the results that the valine content in the extruded blends from fermented and sprouted seed blends increased significantly with sesame addition when compared to that of R_{100} (1.78 ± 0.09 g/100 g protein). Similarly, sesame addition indicated significant increase in the histidine content of the extruded blends when compared to that of the control (0.67 g/100 g protein). With regard to phenylalanine, a similar trend of decrease in phenylalanine content was observed with decreased rice flour in the formulations. Although, these values were statistically higher when compared with the control (2.30 g/100 g protein), statistically higher values were recorded in extrudates produced from the sprouted seed blends when compared to the fermented counterparts.

This increase is in agreement with the report of Ikram *et al*. (2021) who suggested that sprouting brown rice greatly increases the phenylalanine content and some other basic amino acids. The arginine content of the extruded blends also significantly increased with increased sesame addition in both formulations of fermented and sprouted seed extrudates which could be due to the addition of sesame flour. The threonine content of the extruded blends was significantly higher when compared to 2.17 g/100 g protein recorded in the control (R_{100}). This increase could be due to degradation of storage proteins into FAA, during fermentation and sprouting of the seeds (Ohm *et al*., 2016). Ikram *et al*., (2021) reported a high increase in the basic amino acids lysine, leucine, isoleucine, threonine, and valine as a result of sprouting which also increases both the solubility and digestibility of proteins. The Tryptophan content was statistically higher in the extruded blends as compared to the control (0.93 g/100 g protein). Several studies including that of Filli *et al*. (2011) reported that addition of soybean flour during the production of extruded fura resulted in higher essential amino acid content. Singh *et al*., (2007) also showed that mild extrusion conditions enhance the nutrient contents including the amino acids and some physical characteristics, whereas high extrusion conditions associated with high screw speed (> 250 rpm), low moisture (< 20 %) and/or improper feed blend compositions affect the nutrient contents of extruded products. In contrast to this study, Anuonye *et al*. (2010) found a significant reduction in essential amino acids in extruded acha-soybean blends. The essential amino acids of the extrudates observed in this study were in sufficient quantities to meet the requirements of adults while children requirements are also substantially present. Thus, fermentation and sprouting of rice and sesame flour blends show a great potential for utilization in developing different products made from conventional flour sources that could meet the nutrient requirements of vulnerable population.

4.2.6 Antinutrient composition of extrudates

The antinutrient (tannin, phytate and oxalate) composition of the extrudates indicated a slight increase in tannin contents in the fermented seeds extrudates while an inverse trend was observed in the extrudates produced from the sprouted seeds blends. According to Eze *et al*. (2020), tannins are polyphenols, and all polyphenolic compounds are water-soluble in nature. This difference could be accounted for by different processing methods such as differing steeping times and freeing of bound minerals during fermentation and sprouting respectively. The increase in tannin during fermentation could be attributed to hydrolysis of condensed tannins such as proanthocyanidin to phenols. Although there are no safe levels for food antinutrients, the tannin values obtained in this study were significantly lower than the value reported by Eze *et al*., (2020) when sorghum and charamenya flour blends were extruded. Tannins form insoluble complexes with proteins, thereby decreasing the digestibility of proteins. They have also been found to decrease palatability, cause damage to the intestinal tract, and enhance carcinogenesis (Kumari and Jain, 2012). The results of phytate content in the extrudates suggested an increase in phytate content with increase in sesame flour in the blends.

The significantly ($P < 0.05$) lower values recorded in the extrudates produced from blends of the sprouted seeds may be attributed to the increase in phytase activity which leads to hydrolysis of phytic acid during germination (Kumar and Anand, 2021). Although these values were higher than (0.53 mg/mL) recorded for the control (R_{100}), the results obtained from this study were significantly lower than 3.60 mg/mL reported by Anuonye *et al*. (2010) for extruded acha/soybean blends. This could be due to the initial processing methods and the phytate contents of the cereal/legume used. It would be expected that lowering the phytate content should enhance bioavailability of minerals like zinc and iron in the extrudates. This is expected as phytic acid has been implicated in making certain minerals unavailable, as reported by Eze *et al*. (2020). From the results of the oxalate content in the extrudates, a significant ($P < 0.05$) increase was observed as sesame flour increased in all the blends. Akusu *et al*. (2020) reported 87.47 mg/100 g of oxalate in raw sesame seeds. Therefore, fermentation and sprouting greatly reduced the oxalate contents as observed in the extrudates. The relatively lower values seen in the fermented blends could be due to dehulling of sesame before fermentation.

4.2.7 Functional properties of extrudates

The bulk density (BD) of extrudates decreased significantly ($P < 0.05$) with the incorporation of sesame compared to the control (3.10 ± 0.19). Breakdown of starch during fermentation and sprouting could reduce starch content and decrease the bulk density. According to William *et al*. (2018), BD is a measure of flour heaviness and an important parameter for determining packaging requirements, material handling and application in the food industry. The BD observed in this study could be related to homogeneous protein matrix of the developed rice–sesame flour blends with compact or no air cavity layers, making it nonspongy upon hydration. Higher values of bulk density

(2.45 and 2.45 g/ml) were reported by Agunbiade and Ojezele (2010) for breakfast cereals made from maize, sorghum, African yam beans and soybeans. The low bulk densities recorded in this work could be advantageous in infant formulation. The water absorption capacity (WAC) values increased significantly ($P < 0.05$) with increase in sesame incorporation in both fermented and sprouted seed extrudates.

There was significantly lower WAC in the control sample (2.32 ± 0.14 %) which might be due to less polar amino acids and due to the association of amylose and amylopectin in the starch. WAC is an indirect measure of starch digestibility that depends on the extent of gelatinization and dextrinization of starch components (Pardhi *et al*., 2019). Higher WAC indicates higher protein content in the formulations, which absorbs and binds with more water. Although, these results are not in conformity with that of Nwezeh and Ndaliman (2018) who reported higher water absorption capacity in millet and pigeon pea extrudates (4.10 – 5.69). Scientific research has shown that high WAC indicates that food samples hold a large volume of water during cooking into gruels, to yield voluminous low energy and nutrient food (William *et al*., 2018). The hydrophilic nature of the developed blends determines its interaction with water molecules. The higher WAC of extrudates suggests that the extrudates will perform better in food requiring paste preparation (Yusufu *et al*., 2018). Significantly ($P < 0.05$) lower values of SC were recorded in the fermented blends when compared to the control (4.77) while higher values were recorded in extrudates produced from the sprouted flour blends except $SR_{50}S_{50}$ (4.30) when compared with the control (4.77). The SC is influenced by the particle size, species variety and method of processing or unit operations (Awuchi *et al*., 2019). It is an indication of the noncovalent bonding between the molecules of starch granules and also one of the factors of the amylose and amylopectin ratios (Iwe *et al*., 2016). High starch content increases SC of foods and flours, especially in starch with higher amount of the branched amylopectin. The variation observed between extrudates from fermented and sprouted flour blends could be attributed to the methods of processing and unit operations involved.

There was significant increase in the SP values when compared to that of the control (2.00) except in the extrudates produced from equal proportions ($FR_{50}S_{50}$) of fermented rice and sesame flours (0.89). Significantly ($P < 0.05$) lower values (1.52 and 1.06) were recorded in samples $SR_{60}S_{40}$ and $SR_{50}S_{50}$ when compared to that of the control (2.00). High starch content increases SP of foods and flours, especially in starch with higher amount of the branched amylopectin. Several authors including Jie Xu *et al*., (2012) reported that starch swelling power is mainly influenced my amylopecting content. The significantly ($P < 0.05$) lower values observed in $FR_{50}S_{50}$, $SR_{60}S_{40}$ and $SR_{50}S_{50}$ may be attributed to the reduction in rice flour which in turn reduces the amylopectin contents in the formulation. Water solubility index (WSI) is the measure of the degree of starch degradation during food processing which is reflected in the amount of polysaccharides from starch component (Nahemiah *et al*., 2017). Flour solubility is one of the functional properties usually determined during the development and testing of a new flour or flour composite. There was significant decrease in WSI within and between the extrudates produced from both treatments and the control sample (7.98). The presence of lipids reduces water absorption capacity of foods (flours) which can lead to reduced swelling capacity and consequently reduced solubility (Oppong *et al*., 2015). The significant decrease observed within the extrudates therefore, could be due to increased sesame addition and reduced starch which in turn increases the lipid concentration and consequently, reduced WSI. Pressure, concentration, temperature, and the polarity of a solvent are the main factors which affect the solubility of flour and food components. Artz *et al*. (1990), Badrie and Mellowes (1991) and Nahemiah *et al*. (2017), reported similar observations, where they all agreed that at high temperature and low moisture, long chain molecules of food materials are broken down during extrusion to simple soluble molecules which increases WSI.

4.2.8 Sensory evaluation of extrudates

The sensory attributes of extrudates evaluated showed that the mean scores for appearance, taste, aroma, mouthfeel, aftertaste and overall acceptability varied between the extrudates produced from composites of fermented and sprouted rice-sesame blends. Appearance and taste are important sensory attributes that affect the acceptability of food products. Although the results show a similar trend of a significant ($P < 0.05$) decrease with increase in sesame addition in both treatments, higher values were recorded in the extrudates produced from fermented rice/sesame flour blends. Generally, extrudates from both fermented and sprouted flour blends had significant lower appearance values when compared to the control (7.35). This could be attributed to the respective change in color observed when sesame was added to the rice flours. From the results, taste perception by the panelists increased as the level of sesame flour increased when compared to that of the control (5.06). This could be attributed to the slightly sweet taste of sesame. Significant ($P < 0.05$) increase was also observed in the aroma of extrudates produced from both fermented and sprouted rice/sesame flour blends when compared to the control (4.22 ± 0.13). Iwe (2010) suggested that taste and flavor are physiologically and physically connected with one another depending on the respondents. Similarly, results from this work indicated that taste and flavor of the extrudates were enhanced with increased sesame substitution in the extrudates. This could be as a result of Maillard reaction which is a chemical reaction between amino acids and reducing sugars that gives browned food its distinctive flavor when cooked at high temperatures. From the results, a similar trend was observed in the mouthfeel of extrudates from both fermented and sprouted seeds flour blends with a significant increase ($P < 0.05$) with increase in sesame addition. This result is in agreement with report of Iwe (2010) who observed the increase in mouthfeel when cereal was blended with legumes. Generally, the aftertaste of the control was higher than that of the extrudates produced from both fermented and sprouted seeds. The low aftertaste observed in the extrudates could be attributed to the non-use of basic ingredients such as sugar, milk, butter, etc. Furthermore, a close range of general acceptability was observed between the extrudates produce from both fermented and sprouted seed blends with 30, 40 and 50 % sesame substitution, the highest mean scores for general acceptability (6.95 and 6.60) were seen in $FR_{60}S_{40}$ and $SR_{60}S_{40}$. The results therefore, implied that fermented and sprouted sesame flour could be incorporated in extruded snacks production with up to 50 % substitution without fear of product rejection by consumers.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this current research, the fermented and sprouted rice and sesame blends significantly ($P < 0.05$) improved the proximate composition when compared with that of the untreated rice sample. The moisture, fat and protein contents of the blends reduced significantly ($P < 0.05$) after extrusion, however, the vitamins, mineral content and essential amino acids all increased significantly ($P < 0.05$) when compared to that of the untreated rice sample. There was a variation in antinutrients (tannin, phytate and oxalate) analysed for extrudates from both fermented and sprouted rice-sesame blends. These variations may be a pointer to the different processing methods used.

The functional properties significantly improved with sesame addition in both set of treatments while extrudates with 40 % levels of sesame substitutions were enhanced in terms of general acceptability (6.95 ± 1.09 and 6.60 ± 0.50) for the fermented and sprouted extrudate samples respectively. Consequently, the extrudates from fermented and sprouted rice-sesame blends could serve as sources of nutrients for the production of convenient complementary foods to overcome protein-energy-malnutrition among the vulnerable groups.

5.2 Recommendations

1. It is recommended that extrudates produced from the fermented and sprouted rice-sesame blends should be scaled up for local and commercial purposes to enable the utilization of the products by children and adults as ready-to-use therapeutic diets.
2. Further studies should be conducted on the effect of the extrusion conditions on these products and evaluate the nutrient digestibility of the products.

5.3 Contribution to Knowledge

This current research has contributed to knowledge by showing the;

1. Proximate compositions of different fermented and sprouted rice-sesame formulations.
2. Nutrient and antinutrient compositions of extrudates from fermented and sprouted rice-sesame blends.
3. Functional properties and sensory attributes of the extrudates produced from the different blends of fermented and sprouted rice and sesame seeds.

Consequently, the present research has provided information on the potentials of fermented and sprouted sesame to serve as a source of nutrients for the production of convenient complementary foods to overcome protein-energy-malnutrition among the vulnerable population.

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