Production of *Miso*-like Products using Local Grain Legumes

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Declaration

This thesis is the result of research work undertaken by Dennis V. Gbeddy of the Department of Nutrition and Food Science, University of Ghana, under the supervision of Dr. Kwaku Tano-Debrah.

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ABSTRACT

Miso is a semi-solid fermented food made commonly in Japan from soybeans alone or from a mixture of soybeans and rice or barley. This was a study to determine the suitability of local grain-legumes in the production of *miso*-like products. A traditional two-step fermentation process was adapted to process peanuts, cowpea and soybeans into the *miso*-like products. Three products were formulated with the legume combinations of peanut-cowpea, peanut-soybean and cowpea-soybean in ratios of 1:1. Rice *koji* was prepared with spores of *Aspergillus oryzae*. *Miso* sample obtained from Japan was used as an inoculum. The mixtures were fermented for a period of 60 days at 35°C The moisture content and pH values of the fermenting mass decreased whilst the total acid, lipid, free amino nitrogen, total soluble nitrogen and protein contents increased with fermentation time. These increases were very rapid during the first 10 days of fermentation. Major groups of microorganisms involved in the fermentation were mold, yeast and lactic acid bacteria. Although the total plate counts varied during the fermentation period, the final microbial populations were not significantly different from the initial counts. Total microbial population of the three products varied in the range of 10^4 - 10^6 cfu/g. The highest count was observed in the cowpea-soybean sample, 2.2 x 10^6 cfu/g after 4 days of fermentation. The main microorganisms isolated from the fermenting mass were of the genera *Streptococcus*, *Staphylococcus*, *Micrococcus* and *Pediococcus*. The dominant species that persisted throughout the fermentation period were the *Micrococcus* and *Pediococcus* species. The mold population decreased while that of the yeast and lactic acid bacteria increased initially before gradually coming down with fermentation time. Proximate composition of the dehydrated samples were in

the range of 4.4 - 5.9 % for moisture, 24.9 - 32.6 % for protein, 10.5 - 32.2 % for total lipids, 19.5 -25.0 % for ash, 1.17 - 1.3 % for fiber. Total carbohydrates as determined by subtraction were in the range 13.7 - 25.1 %. The highest amount of protein, ash and reducing sugars were found in the cowpea-soybean product. Functional properties of the products were found to be suitable for formulating into soups and stews. Water absorption capacity was in the range of 557 - 793 g/kg, fat absorption 439 - 532 g/kg, swelling capacity 12.5-29.4 %, foaming ability 1.5 - 5.5 %, emulsifying capacity 57.8 - 67.4 % and emulsion stability of 58 - 65.4 %. All the three products remained very stable at room temperature when stored over a period of 12 weeks. The amount of free fatty acids (FFAs) and peroxides (PV) formed in the samples during the 12-week storage were below 0.4 % and 4 mEq/kg, respectively. Thiobarbituric reactive substances (TBA) formed in the peanut-cowpea sample was in the range of 0.006 - 0.02 mg malonaldehyde/kg of sample. The TBA values decreased significantly with storage time. Sensory analysis revealed the cowpea-soybean as the most acceptable of the three samples. The observed changes and characteristics of the products suggest that local grain-legumes may be suitable for processing into *miso*-like products.

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CHAPTER ONE

INTRODUCTION

1.1 Grain legumes

The word 'legume' is derived from the Latin word 'Legumen' which means seeds harvested in pods. Legumes were among the earliest food crops cultivated by man. Their history as cultivated crops goes back to the neolithic times, when man was passing from hunting and food gathering into the food producing stage of the human development (Aykroyd and Doughty, 1982).

Leguminous seeds are sometimes referred to as 'grain legumes' in current literature. The term 'food legumes' is used to cover both the immature pods and seeds as well as the mature dry seeds used for human food, while it excludes oil bearing legumes and those used for forage and pasture. In present FAO practice, the word legume is used for all leguminous plants, with the seeds being defined according to their fat content. For those containing small amounts of fat, for example broad beans, peas, lentils, etc., the word pulse is used. Seeds containing high proportion of fats, for example peanuts and soybeans, are termed leguminous oil-seeds.

Legumes are cultivated throughout the world. The dry mature seeds, which have a high food value and store well, play an important part in the diets of many people in the world. The seeds are second only to cereals as a source of human and animal food

(Aykroyd and Doughty, 1982). The major grain legumes produced locally (in Ghana) include the different varieties of cowpea and peanuts. Soybean and bambara groundnuts are also produced but on a very small scale. Table 1.1 shows the production of pulses and peanuts between the periods 1989 - 1998 in Ghana.

Table 1.1 Production level of pulses and peanuts in Ghana (1989 - 1998)

Legume (MT)	1989 - 91	1996	1997	1998
Pulses	16,000	15,000 (F)	16,000 (F)	16,000 (F)
Peanut	127,000	133,000 (F)	135,000 (F)	135,000 (F)

Source: FAO Production yearbook. Vol. 52, 1999
MT = Metric tons
(F) = FAO estimate

1.2 Nutritional quality of legumes

Grain legumes were recognized to have the highest protein content among all seeds (Abiose *et al.*, 1982). They have served as a major source of protein for humans especially in the regions where animal protein is scarce. About 20% of proteins currently available to man are derived from food legumes in developing countries (Reddy *et al.*, 1986). Protein content of the different types of legumes varies from about 12% for some cultivars of chickpea (*Cicer arientum*), to over 35% in high protein cultivars of winged bean (FAO, 1989). Table 1.2 shows the proximate composition of some local varieties of grain legumes.

Table 1.2 Proximate composition of local varieties of grain legumes

Grain-legume	Moisture	Protein	Fat	Carbohydrate	Ash
Peanuts	4.9	22.7	48.1	22	2.3
Cowpea	9.3	21.8	1.3	64.8	2.8
Soybean	11.9	36.8	19.2	34.5	4.8

Source: Eyeson, K.K. and Ankrah, E.K., (1975) Composition of Foods commonly used in Ghana (CSIR)

Proteins are not all equally effective in improving growth (FAO, 1989). Protein efficiency depends on the composition and structure of the protein. Proteins from different sources may reinforce one another. Protein quality is a term used to express the effectiveness of a protein in promotion of growth. It is influenced by the amount of essential amino acids in the protein to meet human requirements. Bean proteins are generally rich in lysine, an amino acid often deficient in cereal protein. This is an important factor in the use of legume proteins to supplement cereal-based diets. Table 1.3 shows the percentage of utilizable protein of a cereal fed alone and from mixtures of 90% cereal + 10% bean.

Table 1.3 Percent utilizable protein of cereal fed alone and from 90% cereal + 10% bean mixtures

Protein source	Protein in diet (%)	Utilizable protein (%)
100% rice	6.9	4.10
90% rice + 10% beans	7.9	4.96
100% maize	8.5	2.41
90% maize + 10% beans	10.3	4.10
100% sorghum	7.7	2.23
90% sorghum + 10% beans	8.6	3.93
100% whole wheat	11.0	4.26
90% wheat + 10% beans	12.0	5.94

Source: Bressani, R. 1975. Legumes in human diets and how they might be improved. In nutritional improvement of food legumes by breeding. PAG. New York, John Wiley.

1.3 Utilization of legumes in Ghana

One of the important differences in the use of legume seeds for food between the tropics and the rest of the world relates to the physical forms in which they are used and the procedures employed in preparing them for food. The differences are related in part to the ethnic background, customs and traditions (Aykroyd and Doughty, 1982). This has resulted in every culture evolving its own blend of complementary proteins. Locally, peanuts, cowpeas and bambara groundnuts are the most commonly used legumes and these are often combined with cereals before consumption. Cowpeas are processed into bean cakes and eaten with breakfast porridge, boiled with rice and eaten with foods like plantain and/or gari. Peanuts are roasted or boiled and

made into stews. Lately, soybeans and winged beans have been introduced but despite their high nutritional qualities, they are yet to be successfully incorporated into the local Ghanaian diet (Orraca-Tetteh, 1973). Currently, soybeans have still not been successfully incorporated into the local diet.

1.4 Limitations in utilization of legumes

There are a number of factors in uncooked legumes that contribute to the problems of digestibility and flatulence associated with legumes (FAO, 1989). These factors may be divided into heat-labile and heat-stable factors (Table 1.4.). Examples of these factors are: (i) enzyme inhibitors, which may reduce digestibility if not completely inactivated during cooking; (ii) haemagglutinins, compounds that, appear to reduce the efficiency of micronutrient absorption; (iii) antivitamins, found in some uncooked beans, e.g. antivitamin E factor in raw kidney beans and antivitamin D activity in the

Table 1.4 Anti-nutritional factors in uncooked legumes

Heat-labile factors	Heat-stable factors
Enzyme inhibitors	Cyanogens
Haemagglutinins	Alkaloids
Antivitamins & antimetals	Other unknown factors
Binding compounds	
Goitrogens	

Source: FAO, (1989) Utilization of tropical foods: tropical beans

oil-seed *Glycine max;* (iv) binding compounds such as phytin which are present in many legumes and which influence nutrient availability, (Phytin is believed to reduce the availability of calcium by binding with the pulse protein and lowering its nutrient efficiency); (v) goitrogens which, occur in raw soybeans, peas, beans and peanuts and are believed to restrict the uptake of iodine in the diet, thus inducing a state of iodine deficiency; and (vi) alkaloids found in many legumes which could also be implicated in lowered nutritional performance.

All of these factors, in addition to prolonged cooking time, possibly contribute to a great extent the low levels of grain legume utilization in Ghana and some other parts of the world. Several research activities have been directed at improving the processing and utilization of legumes for food. The Collaborative Research Support Program (CRSP) currently going on at the Nutrition and Food science Department, University of Ghana, is one of such projects. One of the major activities of CRSP is the development of processes to incorporate cowpea in traditional staple foods (Personal Communication). Another activity is to study the storage characteristics of local blends of cereal and legumes.

1.5.1 Improving the utilization of legumes

Traditionally, there has been a long history of trying to improve the nutritional quality and the utilization of foods through the process of fermentation. The types of fermented foods mainly produced in different parts of the world reflect the culture and customs of the people. In the Oriental countries, legumes are typically processed by fermentation to overcome the antinutritional factors commonly associated with

legumes. Examples of these fermented products include: *natto*, *tempeh*, *sufu*, *kochujang*, *tauco shoyu* and *miso*, all of which are made from soybeans (FAO, 1989). Apart from these, several other types of fermented foods are made and consumed in many other parts of the world, particularly in Africa, Middle East and South America. Examples of these include: (i) *Tarhana* which is a Turkish fermented food made from parboiled wheat meal and yogurt; (ii) *Dosai,* prepared in southern parts of India by the fermentation of black beans and rice; (iii) *Agbelima,* a typical fermented product from cassava that is commonly consumed in many communities along the West Coast of Africa; and (iv) *Leblebi,* a popular legume soup in the Maghreb Counties that is made from slightly fermented chick pea, broad beans and lentils (FAO, 1989 and FAO, 1991).

1.5.2 Legume fermentation in Ghana

The act of legume fermentation is not very popular in Ghana. One of the known leguminous fermented products used locally is called dawadawa. It is commonly used in the northern parts of the country for flavouring of soups. Dawadawa is usually prepared from the seeds of the African locust beans (*Parkia biglobosia*), which are soaked overnight in water, dehulled, steamed and fermented spontaneously for a period of six days (Personal communication). These processes are usually performed manually thus, making the production quite tedious. The strong smell of the final product does not make it readily acceptable to many people, especially those from the southern parts of the country.

Currently, the Unilever Group (an industrial Company in Ghana) is producing dawadawa using soybeans instead of the African locust beans. The product, called Royco Dawadawa, is formed into cubes and well packaged. Royco Dawadawa is currently enjoying a tremendous patronage unlike the traditional Dawadawa. The success of this has created the need to further exploit the fermentation of local grain legumes.

1.6 Rationale for the study

Fermentation as a method of food processing has been documented to increase the acceptability of the products by making them more digestible with the formation of improved flavours. According to Hesseltine (1983) the renewed interest in Oriental fermented foods stemmed from factors such as nutrition, the great enthusiasm for vegetarian and natural foods, the search for less expensive high protein foods, the influence of foreign students studying in the West, the need to expand export markets, need to add products to convenience foods to add zest and flavour, and the interest in the activities of microorganisms used in fermented foods. Abiose *et al.* (1982) stated that the increasing amounts of *miso* imported to the United Kingdom, mainly from Japan stemmed from its popularity in the United States of America. It is popularly sold in "healthfood" or "wholefood" shops. Now giant Japanese food processing companies like Ajinomoto, Kikkoman, Miyako Oriental Foods etc. have established subsidiaries in the Western countries purposely for the production and promotion of the use of fermented foods such as soy-sauce and *miso* in Occidental countries.

For centuries, soybean has been the major ingredient used in the production of *misos*. Currently, there is tremendous interest in the use of other grain legumes, especially in countries where climatic conditions may not be conducive for growing the soft varieties of soybean suitable for *miso* production. The purpose of the study was to explore the suitability of local grain legumes like peanut, cowpea and soybean or their combinations, for the production of *miso*. The success of this study would greatly help to diversify the forms in which these legumes are consumed locally.

The fermentation processes are expected to enhance the utilization of some of these legumes. The flavor enhancing qualities are likely to render dishes prepared from the product more tasty and appealing to the consumer. It would also add to the product base of these food commodities. Consumption of these products formed is expected to serve as a supplementary protein source.

1.7 Objectives of study

The main objective of the study was to develop a process for the production of *miso-like* products using local grain legumes like peanuts, cowpeas and soybeans.

The specific objectives were to:

1. process local grain legumes into *miso*-like products.

2. investigate process characteristics in the processing of local grain legumes into *miso*.

3. investigate the physical, chemical and microbiological changes associated with the fermentation process.
4. investigate product characteristics like the proximate composition , functional properties, storage quality and the acceptability of *misos* produced from the local legumes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Production and consumption of *miso*

2.1.1 What is *miso*

Miso is a popular semi-solid fermented soybean food commonly used in Japan, China and other parts of the Orient (Beauchat, 1978). The word *miso* may also refer to any traditional fermented product containing soybean, cereals and salt (Shieh *et al.*, 1982). This paste-like product is typically used as a soup base. It is often used as a salty meat-like seasoning for vegetable stews, and also as a base for pickling with meat egg and fish Ebine, 1986).

Nutritional quality of *miso*: A variety of *miso* exist; non-salty *miso*, low salt miso and salty *miso*. The biological value of non-salty and low salt *miso*, according to Obata *et al.* (1932) is 73.2 and 70.7 respectively, compared to casein, which has a biological value of 80.1. This makes the nutritional quality of proteins from *miso* almost equivalent to that of milk, which is regarded as one of the best sources of protein available to man. The quantity of unsaturated fatty acids including linoleic and linolenic acids is over 60% in various types of *miso* (Ebine, 1986). About 30% of salty rice *miso* consists of water-soluble components (other than NaCl). These include free amino acids, peptides, free sugars and organic acids, which are all highly digestible. Table 2.1 on (page 22) shows the nutritional composition of various types of *miso*.

Table 2.1: Nutritional composition of various types of *miso*

Component	Sweet *miso*	Salty light *miso*	Salty red *miso*	Barley *miso*	Soybean *miso*
Moisture (%)	42.6	45.4	45.7	44.0	44.9
Protein (%)	9.7	12.5	13.1	9.7	17.2
Fat (%)	3.0	6.0	5.5	4.3	10.5
Carbohydrate (%)	36.7	9.4	16.1	28.3	11.3
Crude fiber (%)	1.2	2.5	2.0	1.7	3.2
Ash (%)	6.8	14.2	14.6	12.0	12.9
Salt (%)	6.1	12.4	13.0	10.7	10.9
Calcium (mg/100g)	80.0	100.0	131.0	80.0	150.0
Phosphorus(mg/100g)	130.0	170.0	200.0	120.0	250.0
Iron (mg/100g)	3.4	4.0	4.3	3.0	6.8

Source: Ebine, (1986) Legume based fermented foods. CRC Press Inc.

Health-promoting qualities of *miso*: Regular consumption of *miso* has been associated with many health-promoting qualities. In a study involving 122,261 males and 142,857 females aged 40 or more in 29 Health Center Districts of Japan between 1966 and 1978, Hirayama, (1981) came out with a conclusion that the daily intake of *miso* soup reduces the mortality rates for gastric cancer patients considerably. Okazaki *et al.* (1983) also found a significant antimutagenic effect in the lipid fraction of *miso*.

2.1.2 History of *miso* production

Miso has been a popular food produced and consumed in the Orient for many centuries. It was originally made and consumed by Monks in the Buddhist Monasteries of China. The earliest known ancestor of *miso* is said to be the Chinese food called "chiang" which, was thought to have originated before the Chau dynasty of 722 – 481 B.C. (Abiose *et al.*, 1982). Historical accounts suggest that *miso* was introduced to other countries in the Orient from China, where most soybean products were speculated to have originated. Since its introduction into other countries, it has been referred to by names such as *jang* in Korea, *tao–tjo* in Indonesia and Thailand, *tao-tsi* in the Philippines (Ebine, 1986).

Miso is said to have been introduced into Japan about 1,300 years ago, most probably by Buddhist envoys from China. Though there have been a lot of ancient writing about fermented soybean foods, the first scientific report according to Hesseltine, (1983) is only about 100 years old. The first Westerner to study *miso*-making process was said to be a German named Otto Kellar who wrote a detailed article on the subject in 1893 after visiting Japan on an exchange program (Shurtleff and Aoyagi, 1976). His article aroused the interest of the scientific world, resulting in the publication of many other papers on fermented foods and drinks. The period of active interest however ended abruptly with the advent of World War 1 when the exchange of students and cooperation between Japan and Germany ceased (Ebine, 1986).

2.1.3 Processing of *miso*

The first known varieties of *miso* were speculated to have been made from fish, shellfish and game (Shurtleff and Aoyagi, 1976). The earlier methods of preparation had since changed over the years where, the raw materials and production methods have seen many modifications giving rise to a large number of variations. After the initial period, the act of *miso* making in Japan was changed drastically by employing the use of rice *koji* (a bloom of mold growing on rice substrate). This created a product with characteristic and unique flavour to the Japanese.

Several methods evolved for the production of *miso* after the initial introduction of the rice *koji*. The particular method employed depends on the raw materials, the preferred taste or the region where the product is being made. A typical home-based preparation was described by Ebine, (1986) to include pounding and mashing of cooked soybeans, shaping it into balls, wrapping the balls in rice straw, hanging the wrapped material under the eaves, and mixing the dried balls with salt and water. Since 1645, when the industrial production of rice *miso* began, new methods have evolved where modern machines were developed to perform many of the tasks that were hitherto done manually. The current methods of *miso* production basically, involve a two-step fermentation process. The first step is the production of a *koji* (mold-cultured steamed cereals or beans). During the second stage, the *koji* is combined with a mixture of steamed soybean, with the addition of salt and a culture of mixed lactic acid bacteria and yeast. These two steps are basic to the production of all types of *miso,* but the duration of the brine fermentation stage and the raw materials determine the variety of

miso being made. The duration of the brine-fermentation stage varies from two weeks for sweet *miso*, to about three years for some varieties of soybean and salty rice *miso* (Ebine, 1986). Traditionally, brine fermentation is carried out at ambient temperature and the duration extends from three months onwards for the processing of salty rice *miso*. In some areas, the mixtures are filled into concrete tanks placed in a shade. The rhythmic changes between the day and night temperatures have been speculated to improve the *miso* flavour. In industrial production, the fermentation is carried out in air-conditioned rooms where the temperature could be controlled. The conditions as well as temperatures used during various stages of production are often kept as industrial secretes. The fermentation period for ripening also varies from one type of preparation to the other. It typically ranges from 10 to 14 days in sweet *miso*, and to 3 to 4 months for salty *miso* at a temperature of 30 °C.

2.1.4 Types of *miso* and their raw materials

Since the introduction of *miso* into Japan, there have been many modifications, especially in terms of the raw materials used and the methods of production. This has led to the creation of numerous varieties of *miso*, which, have been classified, into different groups by different authors. The three main groups according to Abiose *et al.* (1982) are rice *miso* which has the *koji* prepared from polished rice, barley *miso* has the *koji* prepared from barley and beans *miso* with the *koji* prepared from either soybeans or soybean-wheat mixture. These three main groups of *miso* have also been recognized by Hesseltine and Wang (1967) and Watanabe *et al.* (1974). Within these groups are many more varieties, which are classified, based on their taste and appearance (Table 2.2).

The preferred type of *miso* varies from one geographical zone to another. In Japan, the ratio of volume of production of the rice to barley to soybean *miso* is 80:11:9 (Watanabe *et al.*, 1974). According to Ebine (1986) there are other *miso*-like products which include: *kinzanji miso* (made from wheat, soybeans, salt and several varieties of vegetables); *hama-nato* (which consists of granular salty fermented soybean nuggets); *doenjang* (a fermented soybean product in the form of a paste).

Table 2.2 Common varieties, taste and colour of *Miso*

Variety	Taste	Colour
Rice *miso*	Sweet	White
		Red
	Medium salty	Light yellow
	Salty	Light yellow
		Red
Barley *miso*	Sweet	Light yellow
	Salty	Red
Soybean *miso*	Salty	Red

Source: Reddy *et al* (1986).

Rice *miso* is however, the most typical variety produced in Japan. It accounts for about 80% of the total *miso* production in Japan. Rice *miso* is further subdivided by Shibasaki and Hesseltine (1962) into four types, namely, White, *Edo Sendai* and *Shinshu*, based on the relative proportions of raw materials used, the length of fermentation time and the locality in which they are preferred. The different types of

miso differ in flavour, colour, texture and aroma as shown on (Table 2.3). To the user, no two types of *miso* taste the same, they are as varied as the world's fine cheese and wines (Shurtleff and Aoyagi, 1976).

Soybean has been the major legume used in the processing of *miso* but not all types of soybeans are suitable for making *miso*. The preferred variety of soybean for processing *miso*, is the soft, yellow type with high water absorbing capacity, high carbohydrate and low calcium content (Ebine, 1986).

Table 2.3 Conditions for producing different types of *Miso*

Type	Colour	Nature	Ratio of beans: grains: salt (w/w)
White	-	Sweet	10: 10-20: 2.5-3.5
Edo	Pale red brown	Sweet	10: 8-10: 0.25-0.4
Sendai	Red brown	-	10: 4: 0.5
Shinshu	Yellowish brown	Salty	10: 5: 4.5
Barley		Salty	10: 15: 5
Mellow barley	-	Sweet	10: 10: 5
Mame	Deep red brown	Salty	10: - : 2.5

Source: Abiose, *et al.*, (1982) Microbiology and Biochemistry of *Miso* Fermentation

As *miso* is now becoming more accepted in the Western countries, research is now being focused on the substitution of other grains and other legumes. According to Abiose *et al.* (1982), the use of indigenous crops is attractive both in the potential

saving on imports and the chance to upgrade materials of marginal utility in human nutrition. Kao (1974) produced *miso* from chickpea (*Cicer arientum*) and horse bean (*Vicia faba*) by substituting these for soybeans. Fukunaga (1973) also used waste liquor from boiled soybeans in the production of *miso*. Shieh *et al.* (1982) produced *miso*-like products from a combination of peanuts and soybeans.

2.1.5 Levels of production and consumption pattern of *miso*

The amount of rice, barley and soybean *miso* produced in Japan in 1982 was estimated by Ebine (1986) as 451,880; 69,220; and 57,200 tons respectively from 180,000 tons of soybean, 600 tons of defatted soybean, 104,000 tons of rice, 25,000 tons of barley and 70,000 tons of salt. *Miso* production in Japan was said to have decreased from 590,000 tons in 1973 to 572,000 tons in 1983. This decrease was probably due to changes in food patterns, i.e., from traditional Japanese to western foods, and secondly due to concerns about the high salt content in *miso* (Ebine, 1986). In earlier times, home-based factories on small scale produced the greater quantity of *miso* consumed. This trend was changed after the Second World War, where *miso* is now produced from well-established factories in large quantities under strict hygienic conditions. In 1980, home-based production of *miso* in Japan was estimated at 72,000 tons. According to Shurtleff and Aoyagi (1883) the total *miso* consumption increased in the U.S.A. It rose from 1,122 tons in 1975 to 2,349 tons in 1982. Data on current production levels could not be obtained.

Miso is used as an ingredient in soups and stews. Soups are prepared by dissolving a

lump of *miso* and condiments into hot water to which previously cooked vegetables, *tofu*, mushrooms, meat, fish and other suitable ingredients might have been added. Hesseltine and Wang (1967) suggested the spreading of *miso* thinly on cucumbers or substituting it for meat sauce on spaghetti. High salt concentrations used for processing of *misos* may make this suggestion not very useful. The daily per capita consumption of *miso* was estimated at 28-30 g during the years from 1951 to 1959, and from then till 1982, it gradually decreased to the level of 17g (Ebine, 1986).

2.1.6 Microorganisms involved in *miso* fermentation

2.1.6.1 Starter cultures

One of the unique characteristics of Oriental fermentation is the preparation of a mixed-culture inoculum produced as the starter for a number of fermentations. These starter cultures are found in many countries in Southeast Asia, China and the Indian subcontinent under names such as Chinese yeast, *murcha*, *ragi* etc. They are made up of molds such as *Amylomyces* and *Rhizopus*; and yeast such as *Saccharomyces fibuligera* and *Saccharomyces malanga* (Hesseltine, 1983). These are often prepared under relatively poor microbiological conditions by persons untrained or poorly trained in microbiology. This came by the selection over the centuries of certain microorganisms that can grow together to produce a desirable product free from toxins and infectious organisms. It may have been done unknowingly by establishing ecological conditions that allow the useful organisms to compliment each other's activities and to live in harmony. In Japan, the main starter culture used for most fermentation has been virtually standardized and this is the *tane-koji*. It basically

consists of rice flour and spores of mold species belonging to the *Aspergillus* family. As a rule it is only *Aspergillus* species that are used for production of *tane-koji*. It is currently produced in form of dry spores in well-established factories

In mixed culture fermentations, the substrate may undergo several different transformations at the same time, the total not being possible for any one organism (Hesseltine, 1983). Thus, in the *koji* starter culture, two or more strains of *Aspergillus* are often mixed, with one strain producing high levels of amylases and the second producing specific protease found in low levels in the first strain. The result is a mixture of desirable enzymes that can act and degrade the substrate.

2.1.6.2 Molds

The major role of *koji* is to supply amylolytic, proteolytic and lipoytic enzymes, which hydrolyze the substrates. Two types of molds namely *Aspergillus oryzae* and *Aspergillus sojae* are the most common found in *koji*. In some instances, only one strain of *Aspergillus oryzae* or *Aspergillus sojae* is used, but in many instances, as in the soy-sauce *koji*, as many as two stains of mold are blended together (Hesseltine, 1983). Shieh *et al.* (1982) experimented with the use of *Rhizopus oligosporus* for the rice *koji* and the resulting product was found to be good. The growing mold releases numerous hydrolytic enzymes that break down proteins and the polysaccharides present in the seeds to monomers and soluble oligomers, which are then fermented by yeast and other bacteria in subsequent anaerobic stages (Hesseltine *et al.*, 1976). Typically, the *koji* mold consumes about 10% of the rice in the production of the enzymes. The enzymatic activity produces low molecular weight compounds, which

are involved in the development of flavours. Some of these also act as nutrients for the microorganisms during the ripening of *miso*.

2.1.6.3 Bacteria

Bacteria species such as *Pediococcus halophilus* and *Pediococcus acidi lactici* are the most dominant lactic acid types, (Ebine, 1986). These organisms can survive in 23% brine, but since their acid tolerance is comparatively weak, they cannot survive below pH 5.0. The role of these bacteria in *miso* fermentation is to produce organic acids from carbohydrates, which lower the pH and accelerate the growth of salt-tolerant yeast. *Pediococcus acidi lactici* can grow under low levels of salt (<10%) and produce lactic acid until the pH comes down to 4.0.

Streptococcus faecalis and *Streptococcus faecium* are the dominant weak lactic acid bacteria, which grow well during *koji* preparation. These produce lactic acid, which prevent the growth of bacillus and clostridium. They however disappear after mixing the *koji* with brines since their salt tolerance is very low.

Micrococcus epidermidis, *Micrococcus varians*, *Micrococcus congromeratus* and *Micrococcus caseolyticus* are the dominant aerobic bacteria that grow well on rice *koji* and produce small amounts of acid. These however decrease rapidly during the second stage of fermentation where *koji* and legumes are mixed with brine.

2.1.6.4 Yeast

Alcoholic yeast fermentation is believed to take place during *miso* fermentation. The yeast is believed to contribute to the development of *miso* flavour. Salt tolerant yeast isolated from *miso* includes *Saccharomyces rouxii*, *Torulopsis versatilis*, and *Torulopsis etchellsii* and these were thought to be involved in *miso* fermentation (Ebine, 1986). *Saccharomyces rouxii* has been extensively studied by Matsumoto and Imai (1973) and reviewed by Yong and Wood (1974), as the main yeast involved in fermentation of *miso*. It could grow in high salt medium with pH 4.0 – 5.0 and ferment glucose and maltose but not galactose, sucrose or lactose. The products of the yeast fermentation give a characteristic flavour to the *miso*.

2.1.7 Salt and water used in *miso* fermentation

Salt acts as a preservative and exerts a selective action on the microorganisms, which grow during fermentation (Yong and Wood, 1974). According to Yong, (1971), salt is necessary to permit the exclusive development of the flavour and aroma-forming yeast and lactic acid bacteria. Generally, any food grade salt with more than 95% purity and containing less than 1 ppm of iron is used for *miso* production (Ebine, 1986). The specification is to avoid the effect of browning by iron. Small-scale *miso* producers often prefer the use of sea salt, which is believed to give better flavor and nutritional value (Shurtleff and Aoyagi, 1976). This type is strongly favoured by Western companies trading in *Miso*. The total composition of salt in *miso* varies from about 5% in some sweet varieties to about 14% in salty red *miso*. In industrial preparations, where only known microorganisms are used, the salt concentration may

be reduced without risking the growth of any pathogenic bacteria. Since the consumption of high amounts of salt is now considered as a serious health problem, research activities are now being geared towards low salt or salt free *miso* production. One such study was carried out by Chiou (1999) substituting the use of salt with ethanol, sugars and polyols. The results were found to be promising.

Soft tap water is normally used for washing, soaking, cooking of raw material and for mixing cooked soybeans, *koji* and salts. The use of hard water containing calcium may not cook the soybeans soft enough.

2.1.8 Composition of *misos*

2.1.8.1 Moisture

The moisture content of *miso* varies from one type to the other. Shibasaki and Hesseltine (1962) observed moisture content in the range of 48-52% and Ebine (1986) 42.6 – 45.7 %. To obtain a moisture content of 48-52%, about 10% water is added to the inoculum at the second stage of fermentation (Abiose *et al.* 1982). The moisture content may include ethyl alcohol and other volatile compounds.

2.1.8.2 Carbohydrates

The predominant carbohydrate in rice *miso* is glucose, although nine others have been identified (Mochizuki *et al.*, 1972). In another study by Hondo and Mochizuki (1979), the following sugars were identified in addition to glucose: isomaltose, fructose,

galactose, galacturonic acid, stachyose, mannose, melibiose, arabinose and xylose. The larger part of the starch is hydrolyzed to glucose. There is evidence to suggest that sucrose is mostly converted to glucose during the later stage of the maturation process.

2.1.8.3 Proteins

Proteins are readily digested at the early stages of fermentation. According to Abiose, *et al.* (1982) *misos* contain about 9 - 15% crude protein. Ebine (1986), reported 9.7 – 17.2%. A large part of the protein is digested by proteases to peptides and amino acids. The major amino acid present is glutamic acid, followed by aspartic acid, glycine, valine, threonine and serine (Hondo and Mochizuki, 1968). The total free amino acid content shows a rapid increase in the first 10 days of fermentation and then a slow increase in the later stages of the process. Glutamic acid being the major amino acid gives a meat-like flavour to the product. The ratio of the amino acids changes markedly at the initial stage of fermentation, becoming constant after fermentation for 35 days (Hondo *et al.*, 1969). Table 2.4 on (page 35) shows the amino acid content (total and free) of some varieties of *miso*.

2.1.8.4 Fat

The level of fat in *miso* varies from 3.0 to 10.5% depending on the raw material ingredients and their ratio. Lipids in rice *miso* are gradually hydrolyzed during fermentation to produce glycerol and free fatty acids including linoleic, oleic, palmitic and linolenic acids. These acids can be esterified with ethanol resulting in the formation of characteristic aromas in *miso*.

Table 2.4 Amino acid content (mg/g *miso*) of various types of *miso*

	Edo miso		*Sendai miso*		*Hatcho miso*	
Amino acid	**Total**	**Free**	**Total**	**Free**	**Total**	**Free**
Arginine	8.8	3.5	8.7	3.1	6.8	0.5
Lysine	8.6	2.4	7.4	2.8	9.5	4.5
Histidine	1.5	0.3	1.4	0.3	3.3	0.9
Glycine	6.4	1.4	7.4	2.3	12.6	4.7
Valine	7.5	2.0	8.1	2.2	11.2	5.0
Leucine	11.7	3.2	10.2	4.1	22.8	5.0
Isoleucine	11.5	3.6	11.3	3.6	18.2	7.3
Methionine	2.3	1.2	1.2	0.9	3.7	1.8
Serine	8.6	2.9	6.0	2.0	11.2	5.7
Threonine	4.3	1.4	4.4	1.6	6.8	2.6
Phenylalanine	5.5	1.7	5.9	2.1	6.0	3.3
Tyrosine	4.3	1.5	3.4	1.5	7.1	2.7
Tryptophan	0.9	0.3	1.0	0.2	2.0	0.3
Proline	6.4	2.7	5.8	2.4	10.7	6.4
Aspartic acid	9.6	2.3	5.6	1.8	16.4	4.6
Glutamic acid	20.0	4.9	25.5	6.9	35.2	9.9

Source: Reddy *et al.*, (1986)

2.1.8.5 Organic acids

The main organic acids in *miso* include lactic, acetic citric pyroglutamic, succinic, malic and pyruvic. During fermentation, citric and malic acids decrease while the others increase (Ebine, 1986). The action of yeast on the amino acids during fermentation produces isobutyl, butyl, and isoamyl alcohols that give the *miso* a characteristic aroma. Table 2.5 below shows the organic acid content of some varieties of *miso*.

Table 2.5 Organic acid content (mg/100g) of some varieties of *miso*

Acids	*Hatcho*	*Mugi*	*Shiro*	*Shinshu*
Lactic	165.2	345.4	22.1	64.1
Acetic	30.7	101.5	14.8	102.4
Citric	87.1	70.6	17.8	60.0
Pyroglutamic	109.9	66.2	7.4	5.0
Succinic	81.2	91.2	6.5	54.5
Pyruvic	17.8	22.9	4.1	4.3

Abiose *et al.* (1982)

2.2 Processing and utilization of grain legumes

Legumes are used as food in every part of the world. One important difference in the use of legume seeds for food between the people of the tropics and the rest of the

world is related to the physical forms in which they are used and the procedures employed in preparing them for food (Ihekoronye and Ngody 1985). The differences are related in part to the ethnic background, customs and tradition. Over the centuries, traditional methods of processing and cooking legumes have evolved to give safe, appetising and nutritious products. In many tropical countries, traditional foods are prepared from analogous mixtures of grain legumes, cereals and small amounts of vegetable or animal protein. For example, in West Africa cowpea may be processed into *akara*, a light, crisp-fried product made from well-beaten cowpea paste, flavoured with chopped onions and peppers, and fried very rapidly in deep hot fat; *moin-moin*, is a nutritious tasty dish prepared by mixing cowpea paste with oil, tomatoes paste, finely ground onions and seasonings; *tuubani* a steamed snack prepared from cowpea flour that is commonly eaten in Northern Ghana. Peanuts may be roasted or boiled and made into stews. The introduction of soybeans into communities accustomed to other legumes has encountered acceptability problems relating to its cookability, flavour and its digestibility (FAO, 1989). This is evident in Ghana where soybeans are yet to be successfully incorporated into the local diets despite their high nutritional qualities.

2.3 Enhancing the nutritional quality of grain legumes through fermentation

Fermentation has been one of the oldest and most economical methods of producing and preserving food. It's applications are widespread and various. In many parts of the world, large amounts of fermented foods are produced which served as an essential part of the diet. Scarcely a day passes by in the average Japanese home without fermented soybeans being served at the table in one form or the other (Shibasaki and

Hesseltine, 1962). The types of fermented foods consumed in an area relate closely to the peoples historical background and environment in which they live (FAO, 1991).

The deliberate modification of grains and oil seeds via fermentation with fungi and bacteria has been practiced for many centuries (Shieh *et al.* 1982). Fermented foods may be defined as those, which have been subjected to the action of microorganisms or enzymes so that desirable biochemical changes cause significant modifications to the food. By fermentation the food may be made more nutritious, more digestible, safer or better flavoured (Cambell-Platt, 1987). The preparation and preservation of legumes by fermentation causes certain chemical and physical changes that alter the appearance, body and flavour of the original material (FAO, 1989). These changes invariably improve on the nutritional quality of the product. Nutritional quality of the product may be enhanced by any of the following processes that occur during fermentation:

i. Liberation of nutrients locked into plant structures and cells by indigestible materials. For example, certain molds would breakdown indigestible protective coatings and cell walls both chemically and physically.

ii. Synthesis of several complex vitamins (eg. Thiamine, riboflavin, vitamin B_6, panthothenic acid, etc.) and other growth factors.

iii. Improving the microbial safety of foods. For example, acids and alcohol produced may be inhibitory to common pathogenic microorganisms.

iv. Enhancing the nutritional quality, especially of plant materials, by enzymatic splitting of cellulose, hemicellulose and related polymers not digestible by man into simpler sugars and sugar derivatives.

In addition to the above benefits, fermentation of legumes can either eliminate or reduce to very low levels the enzyme inhibitors, haemagglutinins, antivitamins, antiminerals, goitrogens and cyanides (FAO, 1989). These are all factors often present in legumes that play a contributory role in the problems of digestibility and flatulence commonly associated with them. Increases in tocopherols, which may also occur during fermentation act as antioxidants that help to protect lipids in oil-seed legumes from developing rancid flavours. Gas forming substances as oligosaccharides, raffinose, stachyose and vebarscose are broken down into simpler units to minimize the problem of flatulence. The beany flavours associated with cooked legumes are also reduced drastically. In all, the digestibility of proteins and starches become improved as indicated by increases in water-soluble components like free amino acids, peptides, free sugars and organic acids (FAO, 1989).

Legume-based fermented food products serve as an important source of proteins, calories and some vitamins. Fermentation of the local varieties of peanuts, cowpeas and soybeans is expected to play an important role in the future by providing essential food nutrients and serve as new foods as well. In effect, the fermentation process is expected to help diversify the forms in which legumes are used and would add to the limited vegetarian diets available in this part of the world.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Raw materials:

Samples of peanuts (*Arachis hypogaea*), a local red variety; cowpea (*Vigna unguiculata*), a white variety from Togo; soybean (*Glycine max*), a local cream variety; rice (*Oryza sativa*) 70% broken, (imported from Thailand), and table salt (NaCl) of 99.5% purity were all purchased from the local market. The grains were sorted and sieved to remove all moldy or discoloured ones and extraneous matter from the lot. *Miso* samples and spores of *Aspergillus oryzae* (*tane-koji*) were also obtained from Japan for use as inoculum.

3.2 Processing of raw materials into *miso*-like products

3.2.1 Preparation of raw materials

All the ingredients were properly cleaned as described above and passed through various processing stages before mixing them together for fermentation.

3.2.1.1 Peanuts:

The peanuts samples were placed in a hot air oven at a temperature of 120 °C for 2 h to roast. They were cooled and rubbed between the palms to dehull. The dehulled

samples were soaked in water at room temperature for a period of 18 h. The excess water was drained off and the peanuts cooked to soften in about 50 min.

3.2.1.2 Cowpea:

The cowpeas were dehulled by pre-soaking in water at room temperature for 5 min, drying at 60 °C in a hot air oven for 6 h and passing through a disc attrition mill. The hulls were removed by winnowing. The dehulled cowpeas were roasted in a hot air oven at 120 °C for 1 h after which they were soaked in water for 10 h and cooked for 30 min to soften.

3.2.1.3 Soybean:

Soybeans were dehulled by initially soaking in water for 10 min and drying in a hot air oven at 60 °C for 6 h before running through a disc attrition mill. The grains were broken into coarse grits and hulls removed by winnowing. The dehulled soybeans were then roasted in a hot air oven at 120 °C for 1 h, after which they were soaked in water at room temperature for 15 h, and cooked for 40 min to soften.

3.2.1.4 Preparation of *koji:*

The rice was soaked in water for 15 h, drained and steam cooked for 45 min. It was spread on aluminum trays and allowed to cool down to about 32 °C. After this, the rice was inoculated with *tane-koji* (spores of *Aspergillus oryzae* obtained from the Bio`c Company, Uchida, Japan). The rice was again spread on the aluminum trays

evenly to a depth of about 2 cm, covered with a clean cloth and left to grow for 48 h. The rice was stirred two times during this period. The product formed is called *koji,* (Plate 1)

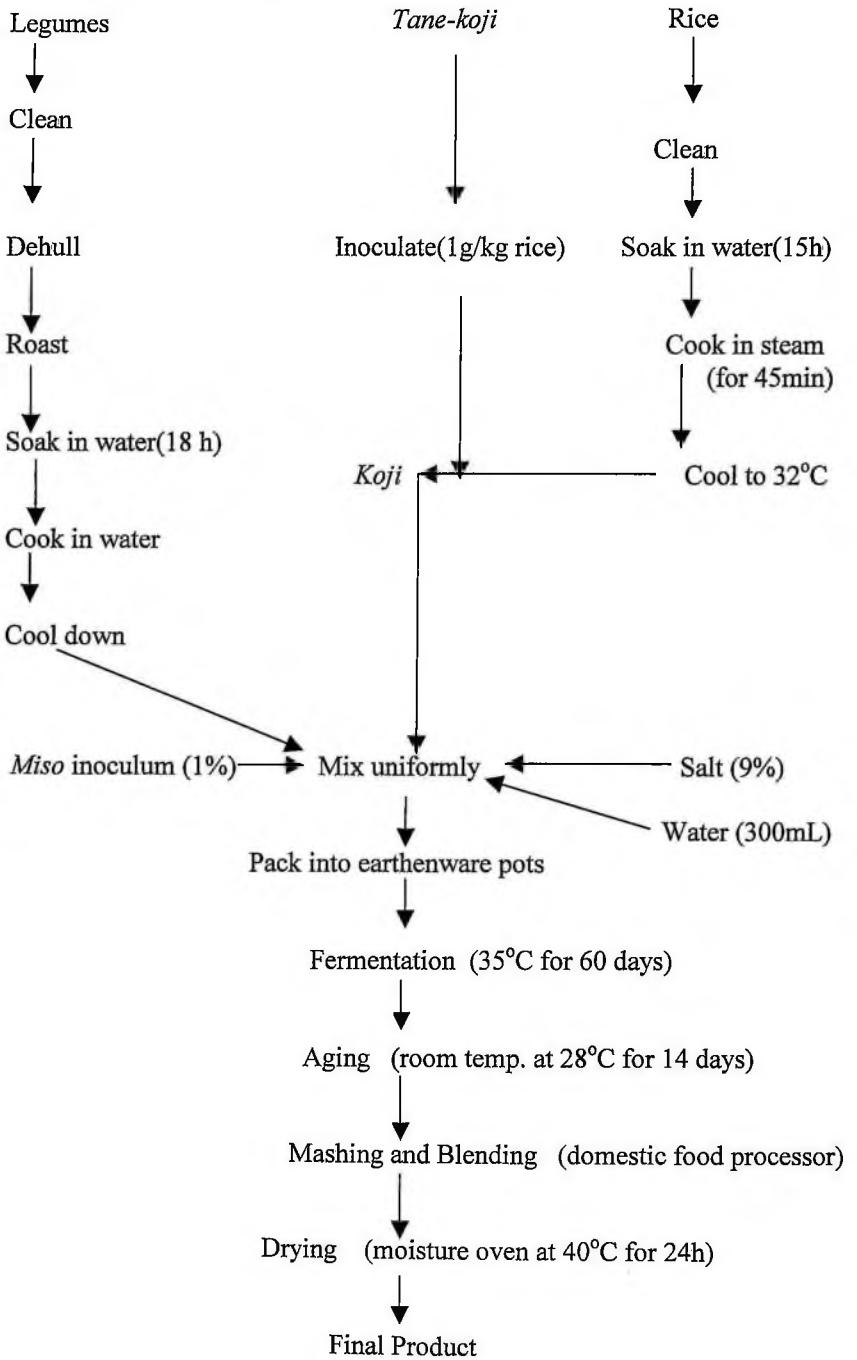
3.2.2 Formulation of products for fermentation

The traditional procedure for the preparation of *miso* was used with some modification as shown on the flow chart in Figure 3.1. Three products, each consisting of two types of legumes and moldy rice were prepared. The preparation involved the mixing of equal amounts of legume and moldy rice. To these were added *miso* samples which have been mixed with table salt and water. The *miso* sample served as an inoculum for the mixture. The proportion of ingredients in each sample were 60 % legume : 30% *koji* : 9% salt : 1% inoculum (*miso* sample). A total of 3 kg of each product was prepared and the formulations were:

Product	**Components**
Peanut-cowpea	Peanut, cowpea, rice *koji*, salt, *miso* and water
Peanut-soybean	Peanut, soybean, rice *koji*, salt, *miso* and water
Cowpea-soybean	Cowpea, soybean, rice *koji*, salt, *miso* and water

Plate 1: Rice *koji* after 48 h of fermentation

Figure 3.1 Process flow chart for the preparation of *miso*



3.2.3 Effect of legume composition on the peanut-cowpea product

The proportion of each legume in the peanut-cowpea product was varied to study their effect on the product characteristics. The proportion of the raw materials used were 60% legume : 30% *koji* : 9% salt : 1% *miso*. Combination of the legumes were as follows:

i. Peanut-cowpea 3:1

ii. Peanut-cowpea 1:1

iii. Peanut-cowpea 1:3

Processing of the raw materials for fermentation was done as described earlier (Section 3.2).

3.2.4 Fermentation of products

The mixtures were pounded in a mortar and pestle into pulp then filled into earthenware pots and covered, (Plate 2). The pots were placed in a room with a temperature range of 27 - 29 °C to begin the fermentation process. On the eight-day, the pots were transferred into an incubator. The temperature was gradually manipulated to attain a temperature of 35 °C in the fermenting mass and kept at this temperature for a period of 60 days. The fermenting mass was transferred into a new pot after 20 and 40 days of fermentation to aerate.

Plate 2: Brine fermentation of products in earthenware pots

3.3 Physico - chemical changes during fermentation

Samples of the fermenting substrates were taken from each product after 0, 4, 10, 15, 20, 30, 40, 50 and 60 days of fermentation to determine moisture, pH, total acid, Free amino nitrogen, total soluble nitrogen and crude protein content of the fermenting mass at the different periods of fermentation. Values were expressed on dry matter basis.

3.3.1 Moisture Content

The Association of Official Analytical Chemists' approved method 925.02 (AOAC, 1990) was used for the determination of moisture content.

3.3.2 pH

The Association of Official Analytical Chemists' approved method 943.02 (AOAC, 1990) was used to determine the pH levels of the fermenting mass. This method was slightly modified by adding 90 mL of distilled water instead of 100 mL. The pH of the solution was determined by the use of a hand held pH Meter (Piccolo Plus, ATC).

3.3.3 Total acid

Total acid was determined according to the method described by Nout *et al.* (1989) as percentage, w/w of lactic acid. After the measurement of pH, the sample was titrated with 0.1 N NaOH to an end point at pH 8.5. The solution was stirred continuously

for 10 min and pH adjusted again with 0.1N HCl for a replicate determination. The amount (mL) of 0.1 N NaOH used to titrate the sample was recorded and total acid calculated as follows:

Total acid (%, w/w) = mL 0.1 N NaOH x 10^{-3} x 90

3.3.4 Total lipid

The total lipid content of the fermenting mixture was determined by the method of Bligh and Dyer, (1959).

Procedure:

Ten grams (10 g) of sample was weighed accurately into a homogenizing flask. After the addition of 10 mL of water, 20 mL of chloroform and 40 mL of methanol, the mixture was homogenized at high speed for 1 min in a homogenizer (ATO Mix - MSE, London). A further 20 mL of chloroform was added into the flask and the mixture homogenized for another 30 seconds. Finally, 20 mL of water was added and the mixture further homogenized for 30 seconds. The homogenate was transferred into glass centrifuge tubes and centrifuged in a Denley BS400 Centrifuge (England) at 2,000 x g for 20 min. After the centrifugation, 20 mL of the chloroform below the aqueous layer was removed by means of a Pasteur pipette into a dried and weighed erlenmeyer flask. The lipid in 20 mL of chloroform was then determined after evaporation, initially on a steam bath and finally in a hot air oven at a temperature of 105 °C for 30 min. The weight of the fat was multiplied by 2 to give the total lipid in the sample. This determination was done in duplicate and the average taken.

3.3.5 Total free amino nitrogen (FAN)

The Colorimetric ninhydrin method was used to determine the free amino nitrogen content of the fermenting mass.

Procedure:

Triplicates of 2 mL of glycine standard solution (107.2 mg glycine dissolved in water and made up to 100 mL), water blank and diluted sample (1:100) were put into three test tubes, respectively. One milliliter of the ninhydrin colour reagent (10.0 g $Na_2HPO_4.12H_2O$ + 6.0 g KH_2PO4 + 0.5 g Ninhydrin + 0.3 g Fructose dissolved in 100 mL of distilled water) was added to each test tube and covered with a glass ball. The tubes were boiled for exactly 16 min. After boiling, the tubes were cooled in a water bath for 20 min and 5 mL of dilution solution (2.0 g KIO_3 dissolved in 600 mL of H_2O + 400 mL of 96% ethanol) added to each. The tubes were mixed by a Vortex mixer and absorbance determined at 570 nm. This determination was done in triplicates and the average taken. FAN was calculated as follows:

$$\text{FAN (mg/mL)} = \frac{(\text{ABS of sample} - \text{ABS of blank})}{\text{ABS of standard}} \times 2 \times F$$

F = dilution factor of sample.

3.3.6 Total protein N

The total N protein was determined by the modified Macro Kjeldahl method 2.049 (AOAC, 1975)

Procedure:

About 2 g of sample was weighed, wrapped in a filter paper and introduced into a Kjeldahl flask. This was done in duplicates. A blank was prepared by introducing some filter paper into another flask. A catalyst, Reese and Williams reaction mixture (0.1 g SeO_2 + 0.25 g $CuSO_4$ + 9.65 g K_2SO_4) was added together with concentrated H_2SO_4 (25 mL). The mixture was digested with the Kjeldahl apparatus to a greenish-blue colour in about 45 min. This was cooled and diluted with distilled water (150 mL) and 50% NaOH solution (80 mL). A few boiling chips were added and the flask arranged on the distillation unit and the heater turned on. Approximately, 150 mL distillate was collected into 100 mL of 2% Boric acid solution. A Conway indicator was added and the distillate titrated against 0.1N H_2SO_4 to a faint pink colour.

Total N = (mL) of 0.1N H_2SO_4 x 0.0014 g

Total protein N = Total N x 6.25

3.4 Microbial analysis

Different groups of microorganisms are involved in the fermentation of *miso*. These are mostly mold, salt tolerant yeast and lactic acid bacteria species. To study the microorganisms active at the various stages of fermentation, 10 g of each product was aseptically transferred after 0, 4, 10, 20, 40 and 60 days of fermentation into sterile stomacher bags and prepared for analysis. The products were homogenized with a Seward Stomacher 80 (BA 7020, England) for 1 min using Butterfield's phosphate buffer solution as a diluent. The products were then analyzed for changes in total microbial population and the groups of microorganisms active at the different stages.

3.4.1 Total microbial population

Total population counts of the mesophilic bacteria isolated were determined using the Total Plate Count method, on a Plate Count Agar (pH 7.0, Biolife. S.r.l., Milano – Italy), supplemented with 3% NaCl. The plates were incubated at 35 °C for 3 days. Plates with (25 – 250) colonies were counted and the number of colonies expressed as colony forming units per gram of sample (cfu/g).

3.4.2 Identification of microorganisms

Typical colonies from all plates were isolated and sub-cultured for purification on Nutrient Agar (Merck, Darmstadt, Germany). The isolated colonies were then cultured on Nutrient Agar slants and stored in a refrigerator until they were identified. The microorganisms were identified using their morphological and biochemical properties such as gram stain, pigment formation and reaction with sugars as described in the Bergeys Manual.

3.4.3 Population changes in the different groups of microorganisms

The changes in the population of the different groups of microorganisms in the peanut-cowpea product were studied by enumerating the population of the molds, yeast lactic acid bacteria as the fermentation progressed.

3.4.3.1 Enumeration of molds and yeast

Potato Dextrose Agar (PDA, pH 5.4 Biochemika, Switzerland), supplemented with 3% NaCl and 100 ppm of chloramphenicol and 100 ppm chlortetracycline-HCl was used for enumerating mold population in the peanut-cowpea samples. The plates were incubated at 30 °C and mold population counted after 2 days, and yeast after 5 days.

3.4.3.2 Enumeration of lactic acid bacteria (LAB)

The populations of lactic acid producing bacteria in the fermenting mass were determined by the use of an overlaid MRS agar medium (de Man Rogosa Sharp, Oxoid Ltd., Hampshire, England). Plates were incubated at 30 °C for 7 days.

3.5 Proximate composition of products

The proximate compositions of the dehydrated products were determined for their nutritional characteristics. Moisture, total lipids, total acid and crude protein content of the products were determined by the same methods as described in (section 3.3). The other parameters determined were ash, fiber and soluble solid contents.

3.5.1 Total ash

The Association of Official Analytical Chemists' approved method 900.02c (AOAC, 1990) was used for the determination of total ash content

3.5.2 Crude fiber (Weende. Wikjstrom Method)

The crude fibers content of the samples were determined by the method of Weende Wikjstrom, (Selecta, 1995).

Procedure:

About 2 g of each product was weighed into a porous crucible and placed in a Dosi-Fiber unit (J.P. Selecta, s.a., model Km595, Barcelona). Two hundred milliliters (200 mL) of preheated sulphuric acid (1.25%) was added to the column, followed by opening of the cooling unit and then turning on the heating elements. This was allowed to boil vigorously for 30 min, after which the column was drained and then flushed with a small amount of preheated distilled water. This process was repeated by the use of 200 mL of KOH (1.25%), followed by 100 mL of HCl (1.0%). The column was again flushed with about 150 mL of preheated distilled water. After this, the crucible was removed, dried in a hot air oven at 150 °C overnight. The crucible was placed in a Muffle furnace at 550 °C for 6 h, allowed to cool down and weighed. The difference in weight was used to determine the fiber content.

3.5.3 Soluble solids in the products

Soluble solid content of the products were determined by a method described by Shieh *et al.*, (1982). The *miso*-like product were mixed with water (2:1 ratio, *miso*:water, wt/vol.) and the amount of soluble solids determined by means of a hand held portable refractometer (Bellingham + Stanley Ltd, U.K) at 27 °C. The dissolved solids were then determined as percentage soluble solids.

3.5.4 Quantity of reducing sugars formed in products

A modified Lane and Eyon method (Whalley, 1964) was used to determine the quantity of reducing sugars formed in the products.

Procedure:

Ten grams (10 g) of dehydrated *miso* sample was dissolved in 100 mL of distilled water, allowed to stand for about 2 h and filtered with Whatman filter paper (no. 4). The sample solution was poured into a 50 mL burette. Exactly 10mL of an equal mixture of Fehlings solution 1 & 2 was pipetted into a 250 mL boiling flask and brought to boil on a hot plate. The sample solution was titrated against the Fehlings solution while still on the hot plate until the blue colour disappeared. Methylene blue solution (1%) was used as an indicator. There was an initial test-run to anticipate the titre value. Three titrations were made and the average taken as the titre value. The percentage of reducing sugars in the sample was determined as follows:

$$\text{Percentage of reducing sugars} = \frac{100 \times 0.1202 \times 100}{\text{sample titre(mL)} \times \text{sample wt.(g)}}$$

3.6 Functional properties of the products

Miso is typically used as a soup base or to flavour stews. To determine the performance of the products in their use for soups and stews some functional properties were evaluated. These include water absorption, fat absorption, swelling ability, foaming ability and emulsifying properties.

3.6.1 Water absorption

About 5 g of sample was weighed and mixed with 20 mL of water and allowed to stand for 30 min. The mixture was centrifuged at 3,000 rpm for 15 min. The amount of water absorbed was measured as the increase in weight of the sample after decanting the excess water.

3.6.2 Fat absorption

Two grams (2 g) of sample was mixed with 5 mL of vegetable oil and allowed to stand for 15 min. The mixture was centrifuged at 3,000 rpm for 15 min. The oil was then decanted into a measuring cylinder. Amount of oil absorbed by the sample was calculated as the difference in volume.

3.6.3 Swelling ability

Ten grams (10 g) was mixed with 100 mL of water in a measuring cylinder and the swell volume measured at intervals of 15 min for a period of 60 min.

3.6.4 Foaming ability

Ten percent (10 %) slurry of the sample was made and whipped in a Warring blender at high speed for a minute and poured into a measuring cylinder. The foam stability

was measured over a period of one hour. Foam capacity was calculated as a percentage of increase in volume.

$$\text{Percentage volume increase} = \frac{\text{Vol. after whipping} - \text{Vol. before whipping}}{\text{Vol. before whipping}} \times 100$$

3.6.5 Emulsifying properties

An amount of 3.5 g of sample was weighed and suspended in 50 mL of water and 50 mL of vegetable oil. The 100 mL mixture was emulsified in a Warring blender at high speed for 1 min. The emulsion was divided equally into two parts in 50 mL centrifuge tubes and centrifuged at 1,300 g for 5 min.

$$\text{Emulsifying activity} = \frac{\text{Ht. of emulsified layer}}{\text{Ht. of whole layer}} \times 100$$

Emulsion stability was measured by heating the emulsion at 80 °C for 30 min. The emulsion was cooled for 15 min in water and centrifuged at 1,300 g for 5 min.

$$\text{Emulsifying stability} = \frac{\text{Ht. of remaining emulsified layer}}{\text{Ht. of whole layer}} \times 100$$

3.7 Storage quality characteristics

Samples of each of the fermented products were divided into three parts. One portion of each product was dried in an oven (Astell Scientific) at 40 °C for 24 h, the second portion mixed with 3% ethanol and the third portion left as a live sample. About 50 g of each portion was sealed in a transparent polythene bag and stored at room temperature (28 - 30 °C) for a period 12 weeks. The levels of Free Fatty Acids, Peroxide Value and Thiobarbituric acid reactive substances in the samples were determined at intervals of 4, 8 and 12 weeks of storage. This was to determine the storage quality characteristics of the samples at room temperature.

3.7.1 Free Fatty Acid (FFA)

Free fatty acids formed in the products during storage was determined as described by Kirk and Sawyer (1991).

Procedure:

A solvent was initially prepared by mixing 25 mL of diethylether with 25 mL of methanol and 1.0 mL of 1% phenolphthalein solution. This was carefully neutralized with a few drops of 0.1M NaOH solution. One gram (1 g) of the sample was weighed and poured into the mixed solvent. This solution was titrated against an aqueous 0.1 M NaOH with constant shaking until a pink colour that persists for 15 seconds is obtained. Four determinations were done for each sample and the mean value taken. The percentage of Free Fatty Acid (FFA) was calculated as follows:

$$\text{Acid value} = \frac{\text{titre (mL)} \times 0.0282 \times 5.61}{\text{Weight of sample}}$$

$$\text{Free Fatty Acid (FFA)} = \frac{\text{Acid value}}{2}$$

(1 mL of 0.1 M NaOH is equivalent to 0.0282 g Oleic acid)

3.7.2 Peroxide Value (PV)

The peroxide formed in the products during storage was determined as described by Kirk and Sawyer (1991).

Procedure:

About 1 g or less of a chloroform extract of the sample was weighed into a clean dry boiling tube, and 1 g of powdered Potassium iodide (KI_2) added to it while the extract was still liquid. Twenty milliliters (20 mL) of a solvent mixture (2 volumes of Glacial acetic acid + 1 volume of Chloroform) was added. The boiling tubes were placed in a boiling water and the liquid brought to boil within 1 min after which it was allowed to boil vigorously for another minute. The boiling liquid was poured quickly into a dried conical flask containing 20 mL of a 5% KI_2 solution. The tube was washed out twice with 25 mL of water and titrated with 0.002 M Sodium thiosulphate solution using starch as the indicator. A blank was similarly performed without the sample. There were four determinations for each sample and the average taken. The peroxide value of the extracted oil was calculated as follows:

$$\text{Peroxide value} = \frac{(S - B) \times M \times 1000}{\text{Weight of sample (g)}}$$

S = sample titration

B = blank titration

M = molarity of Sodium thiosulphate solution

3.7.3 Thiobarbituric Acid (TBA)

The thiobarbituric reactive substances formed in the products during storage period was determined as described by Kirk and Sawyer (1991).

Procedure:

Ten grams (10 g) of sample was thoroughly mixed with 50 mL of water for 2 min and washed into a distillation flask with 47.5 mL of water. Exactly 2.5 mL of 4M HCl was added to the flask to bring the pH to about 1.5, followed by three drops of Octa-1-nol as an antifoaming agent. A few glass beads were added and the flask heated on the Kjeldahl Distillation set-up until about 50 mL of distillate was collected in about 30 min from the time of boiling. The distillate (5 mL) was pipetted into a glass stopper tube and 5 mL of TBA reagent (0.02883 g of TBA / 100 mL of 90% Glacial acetic acid) added. The tube was shaken thoroughly and heated in boiling water for 35 min. A blank was similarly prepared using 5 mL of water and 5 mL of TBA reagent. The tubes were cooled for 10 min in water and the absorbance measured against the blank at 538 nm. There were four determinations for each sample and the average taken. TBA value was calculated and expressed as mg malondaldehyde/kg sample as follows:

$$\text{TBA value} = 7.8 \times (AS - AB)$$

AS = absorbance of sample

AB = absorbance of blank

3.8 Sensory analysis

The newly developed *miso*-like products were tested to evaluate their acceptability. This involved an acceptability and preference test by a randomly selected panel of 30. Appendix 33 shows a sample of questionnaire for the sensory analysis.

Procedure:

Each of the three dehydrated products were used to prepare a soup by mixing an average of 10 g sample in hot water to which previously cooked vegetables (okro, garden-eggs, tomatoes, shallots and cabbage) has been added. This was allowed to boil for about 20 min and then served to the participants while still hot. The samples were coded and each participant requested to grade the soups according to his/her likeness on a 9-point hedonic scale.

Secondly, there was a preference test on the peanut-cowpea product with different proportions of the legumes. The soups were prepared similarly as described and presented to the participants to indicate their preference.

3.9 Statistical analysis

Statistical analysis of the data obtained involved multifactorial and multiple range analysis of variance (ANOVA), using Statgraphics Ver 4.2 (Graphics Software System, STCC, Inc. USA).

ANOVA was done to determine the changes in physico-chemical properties such as moisture, pH, total acidity, free amino nitrogen and total soluble nitrogen with fermentation time.

ANOVA was again used to determine differences in the levels of FFAs, PVs and TBA formed in the products with treatment and store-time during the storage quality characteristic studies.

ANOVA was also used to determine the differences in the acceptability and preference of the products after the sensory analysis was conducted.

ANOVA was done at a significance level of .05% ($\alpha = .05$).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Processing of local grain legumes into *miso*-like products

The traditional two-step fermentation process was adopted for processing the peanut, cowpea and soybean into *miso* with some modifications. The first step involved the *koji* making process and the second, the brine fermentation (Fig. 3.1).

The *miso* processing started with the *koji* preparation. *Koji* is a Japanese word meaning " bloom of mold". The *koji* preparation involved the inoculation of steamed rice with *tane-koji* (dried spores) of *Aspergillus oryzae* and allowing it to grow for a period of 48 hours. According to Hesseltine (1976) the growing mold releases numerous hydrolytic enzymes such as protease, amylase and lipase into the *koji*. The next step was the brine fermentation stage, which involved the mixing of the koji with soft-cooked legumes, an old *miso* sample, salt and water to form the fermenting mixture. These were thoroughly mixed together, packed into earthenware pots, sealed and put into an incubator to ferment.

During the fermentation, the koji enzymes were expected to act on the macromolecules and breaking them down into smaller units. The miso sample added served as a source of Lactic Acid Bacteria (LAB) and yeast. According to Mochizuki, *et al.* (1972) and Ebine (1986), the LAB would act on the smaller molecules to produce organic acids, which lower the pH of the fermenting mixture. The low pH

encourages the growth of yeast that may consequently act on the sugars to produce alcohol and other flavorous compounds. The high salt media would promote the growth of salt tolerant LAB and yeast and at the same time suppressing the growth of any pathogenic bacteria, that enters the fermenting mixture.

Traditionally, *miso* is packed into wooden tabs or concrete tanks for fermentation. During the brine fermentation stage however, the mixtures were packed into earthenware pots for easy handling and control of the processing parameters such as temperature, in the laboratory. According to Shurtleff and Aoyagi (1976) *miso* is traditionally fermented in an open area that is protected from direct sunlight and the mixture allowed to "experience the rhythmic temperature changes of the days and season". This is speculated to improve on flavours in *miso*. From literature, the period of fermentation may extend from a few weeks in sweet *miso* to about 2 years in some varieties of soybean *miso*. Brine fermentation in this study was however carried out at 35°C for 60 days. Fermenting at this temperature, most of the complex substrates were expected to be broken down into smaller units in about 60 days. For example, the proteins were expected to break down into peptides and free amino acids, the carbohydrates into free sugars and the lipids into free fatty acids and glycerol.

4.2 Process characteristics for production of the *miso*-like products

Several processes were involved in production of the *miso*-like products. On inoculation of the steamed-cooked rice with the *tane-koji*, the mold growth became clearly visible after 24 hours. After about 36 hours, the whole surface was covered with a luxuriant moldy growth. Initially the mold growth appeared white but became

slightly creamy after 48 hours when the *koji*-making process was terminated. The rice particles stacked together to form clumps.

One of the important steps in the *miso* making process is the preparation of the raw materials prior to the fermentation process. The peanuts were dehulled by rubbing between the palms after roasting. This process was however found not to be very efficient since some of the hulls remained stuck to the nuts. The cowpea and the soybeans on the other hand were easily dehulled after running through the disc attrition mill. The efficiency of the dehulling process was found to be dependent on how well the grains were dried after soaking in water for varying periods. Roasting of the legumes was done at 120°C. At this temperature, the roasting was thought to be slow. However, it was feared that beyond this, serious deteriorative effects by heat might result. With the product formulation, the peanuts and cowpea were selected based on their popular local use. In an attempt to promote its local use, the soybean was also selected as one of the ingredients. The product of most interest among the three formulations was the peanut-cowpea sample. This was because (i) there was no reported literature of this combination in *miso* preparation and (2) the two commodities are invariably used as soup thickeners in Ghana just as *miso* is used. During the substrate formulation, mortar and pestle was engaged to do the mixing of ingredients, and to partially reduce the particle size of the grain legumes. This was found to be rather tedious. The extent to which this was done was thought to invariably affect the rate of fermentation based on differences in size reduction efficiency. Another critical stage of the *miso* making process is the brine fermentation. In the traditional setting, the mixture is packed into concrete tanks or wooden tabs (as described in section 4.1) and covered with a heavy cover. However, the earthenware

pots used for the fermentation in this study might contribute to the observed moisture loss due to their porous nature. The moisture loss could possibly affect the texture of the final product. The top of the fermenting mixtures became covered with thick creamy bacterial growth after about 10 days and this persisted for most of the fermentation period. A strong fermentative smell of alcohol emanated from the pots from the 10^{th} day onwards. The fermenting mass became darkened in all the samples after 30 days of fermentation. Fermentation was very slow in trial samples where the legumes were not mashed before the fermentation process.

4.3 Physical and chemical changes during fermentation

4.3.1 Moisture content

Changes were observed in the moisture content as fermentation progressed. There was an initial rise in the moisture content of the products during the early stages of fermentation followed by a gradual decrease from the 10^{th} day onwards, (Fig. 4.1). The initial increase in moisture content may be attributed to the rise in microbial activity leading to the breakdown of macromolecules into smaller units with the consequent production of water. After the 30^{th} day, the moisture content of the samples began to decrease significantly ($p< 0.0001$) with fermentation time. There was also a significant difference ($p< 0.0001$) between the moisture content of the three products, (Appendix 12).

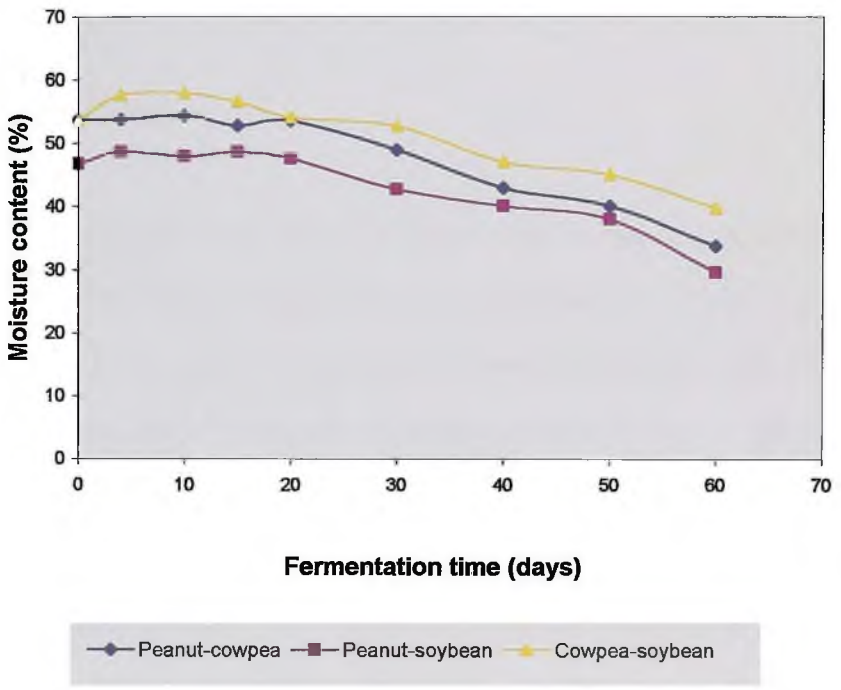


Fig. 4.1 Moisture content of fermenting mixtures

Moisture content of *miso* varies considerably from one to another. This often depends on the substrates and the method of preparation used. *Miso* containing 45% of moisture is considered too hard while 55 % moisture is considered too soft. According to Abiose *et al.* (1982), to obtain the proper moisture level, about 10 % water is added to the *miso* inoculum at the brine fermentation stage. Moisture content is very important to produce a *miso* with a uniform consistency.

4.3.2 pH values

There was a gradual decrease in pH values with the increase in fermentation time for all the three products. The rates of decrease in pH followed a similar pattern (Fig. 4.2). The observed decreases were statistically significant ($p< 0.0001$) with fermentation time. The decreases were mostly evident during the first 10 days as well as between the 20^{th} and 40^{th} day of fermentation (Appendix 13). The fall in the pH values was observed to be highest in the cowpea-soybean sample, from 6.70 – 5.38, followed by peanut-soybean sample 6.77 – 5.49 and that of the peanut-cowpea sample 6.56 – 5.53 (Appendix 2). The observed decrease in pH values of the samples with fermentation time could be associated with the fermentative activity of the lactic acid bacteria (LAB), which produces acid as a result. The pH of *miso* depends considerably on materials and method of production. *Miso* is known to have very strong buffering properties according to Ebine (1986).

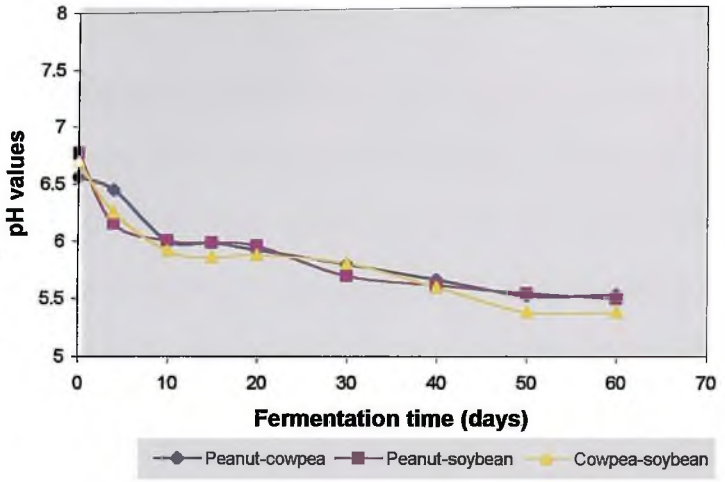


Fig. 4.2 Changes in pH of fermenting mixtures

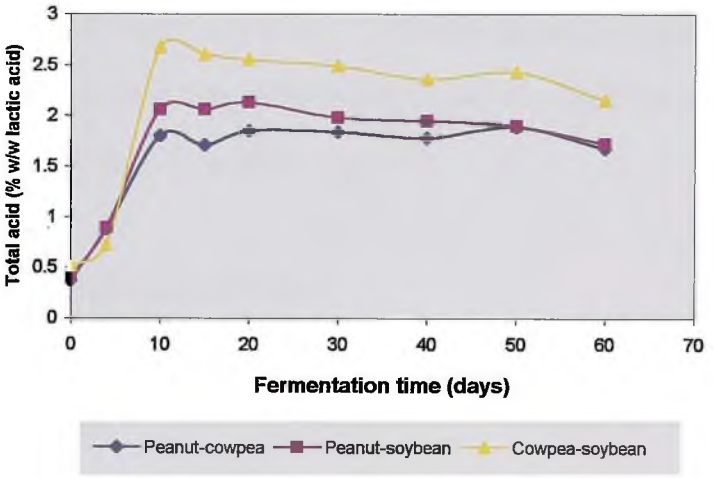


Fig. 4.3 Total acid formed in fermenting mixtures

4.3.3 Total acid (as % w/w of lactic acid)

There were changes in the acidity of the products as the fermentation progressed, (Fig. 4.3). Total acidity of the products increased steadily from the start of fermentation until about the 10th day and stabilized afterwards, with only a slight fall. The initial increase in acidity was found to be significant ($p< 0.0001$). In addition, the amount of acid formed in the cowpea-soybean sample was significantly higher ($p< 0.0001$) as compared to the peanut-cowpea and peanut-soybean samples, (Appendix 14). The highest rise was observed in the cowpea-soybean sample. The range for acidity of the products were: 0.37 – 1.89 % for the peanut-cowpea sample, 0.40 – 2.06 % for the peanut-soybean sample and 0.51 – 2.67 % for the cowpea-soybean sample. The differences in acidity of the products may be related to the differences in fermentable sugar concentrations in the different products.

4.3.4 Total lipid content (% w/w)

The percentage of chloroform-extractable lipid materials increased gradually as fermentation progressed in all the samples for the first 40 days (Table 4.1). The rate of increase was very rapid during the first four days for the peanut-soybean and cowpea-soybean samples and during the first 10 days for the peanut-cowpea sample. After 20 days of fermentation, changes in the extractable lipid content of the samples was minor.

Reports of studies on changes in lipid content of fermenting oilseeds indicated only minor changes during the fermentation period (Yoshida and Kajimoto, 1972). The

results obtained for the above study possibly suggest that lipids become more extractable as fermentation progressed. The increase in extractability could be attributed to the activity of the microbial enzymes. Fullbrook, (1983) observed an increase in the extractability of soybean oil during the enzymatic treatment. Several authors have also reported that enzyme treatment would increase oil extraction from oilseeds (Bhatnagar and Johari, 1987; Sengupta and Bhattacharyya, 1996; Tano-Debrah and Ohta, 1994, 1996). Shieh *et al* (1982) however, attributed these increases to the probable decreases in non-lipid materials rather than to actual increases in lipid content.

Table 4.1 Changes in total lipid content (% w/w) with fermentation time

Fermentation time (days)	Peanut-cowpea Lipid (%)	Peanut-soybean Lipid (%)	Cowpea-soybean Lipid (%)
0	20.6 ±0.42	23.0 ±0.71	9.6 ±1.06
4	24.6 ±1.34	32.2 ±0.99	12.0 ±0.71
10	29.5 ±3.25	33.9 ±0.78	12.4 ±0.64
15	30.9 ±0.64	35.5 ±1.27	12.0 ±1.34
20	30.6 ±1.56	33.4 ±0.28	12.6 ±0.28
30	30.0 ±0.56	32.9 ±0.42	13.5 ±0.56
40	31.0 ±0.71	36.4 ±1.06	14.1 ±0.42
50	28.5 ±0.78	35.9 ±0.21	14.9 ±1.56
60	28.9 ±1.34	33.2 ±0.92	13.7 ±0.35

(on dry matter basis)

The increase in the lipid content was more rapid in the peanut-soybean sample as compared to the peanut-cowpea and cowpea-soybean samples. The peanut-soybean sample showed the highest amount of extractable lipids 36.4 ±1.06% after 40 days of fermentation followed by peanut-cowpea sample 31 ±0.71 % after 40 days and 14.9 ±1.56% for the cowpea-soybean sample after 60 days. The amount of lipid extracted seemed to reflect the oil content of the raw materials used in the product formulation.

4.3.5 Total Free Amino Nitrogen (FAN)

Free amino nitrogen content of all the products increased rapidly during the first 4 days of fermentation after which the rise became gradual up to the 40th day, (Fig. 4.4). After this, the FAN content in all the samples began to fall gradually until the fermentation process was terminated. Total FAN content of the samples increased significantly ($p< 0.0001$) with fermentation time, especially during the first 4 days. FAN content was also found to be significantly higher ($p= 0.005$) in the cowpea-soybean and peanut-soybean samples as compared to the peanut-cowpea sample, (Appendix 15). There was however no significant difference in the FAN content of the cowpea-soybean and peanut-soybean samples.

The initial increases in FAN content with fermentation time could be attributed to the autolytic activities of the *koji* enzymes and the microbial degradation of the grain legumes. This may suggest that the proteins were probably being hydrolyzed gradually into amino acids as the fermentation progressed.

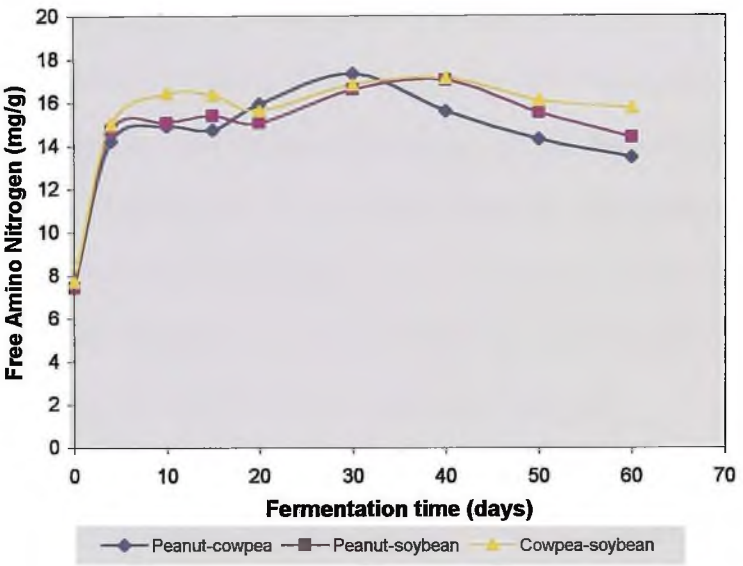


Fig. 4.4 Total Free Amino Nitrogen in fermenting mixtures

4.3.6 Changes in total soluble nitrogen and crude protein contents

4.3.6.1 Changes in Total Soluble Nitrogen

Total soluble nitrogen (TSN) of the fermenting mixtures followed a similar pattern as for the FAN content. There was a rapid rise in TSN during the first 10 days of fermentation in all the three products and after this, the changes became minor (Fig. 4.5). The TSN of the products significantly increased ($p < 0.0001$) during the first 10 days of fermentation, (Appendix 16). Total soluble nitrogen was significantly higher ($p = 0.0004$) in the peanut-soybean and cowpea-soybean products as compared to the peanut-cowpea product. However, the observed difference between the peanut-soybean and cowpea-soybean products was not statistically significant.

The increases in TSN with fermentation time suggest the possible gradual hydrolysis and solubilization of the proteins. This action could be predominantly attributed to the protease activity from the *koji* molds and the breakdown of the polypeptide macromolecules by the microorganisms into smaller units.

4.3.6.2 Changes in crude protein content

Protein content of all products increased appreciably until about the 10^{th} day of fermentation irrespective of the legume combinations (Fig. 4.6).

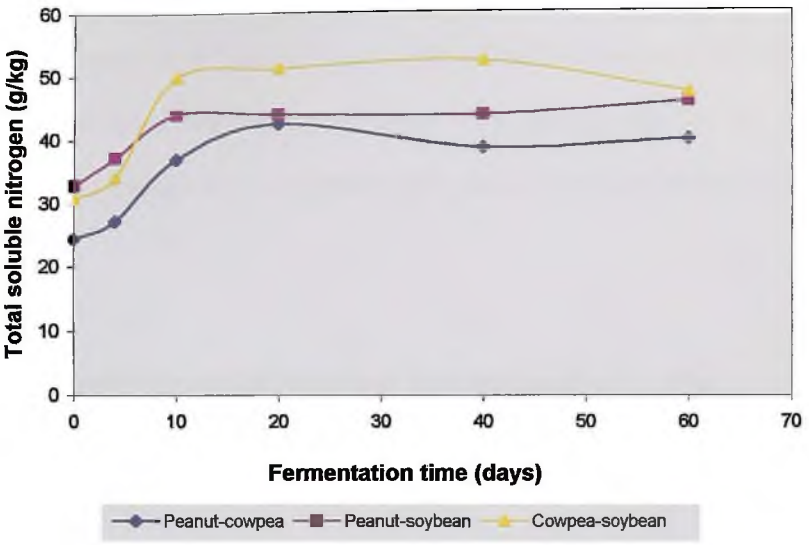


Fig. 4.5 Total soluble nitrogen in fermenting mixtures

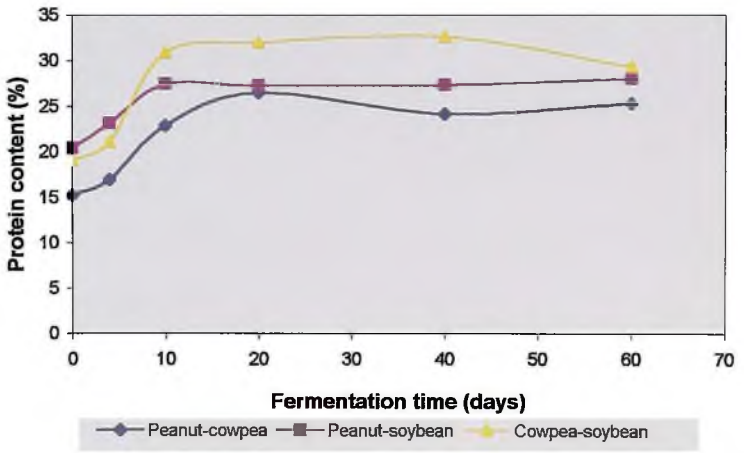


Fig. 4.6 Changes in protein content of fermenting mixtures

Beyond this, the values apparently remained constant until the fermentation process was terminated after 60 days. Protein content of the products increased significantly ($p = 0.003$) between the 4^{th} and 20^{th} day of fermentation, (Appendix 17). The sudden increase in protein content in the early stages of fermentation may be attributed to the rise in microbial mass, which may have contributed to the total nitrogen content of the samples.

4.4 Characterization and identification of microorganisms during fermentation

4.4.1 Total microbial population

Changes in the total microbial populations of the different products during fermentation were as shown in Figure 4.7. Each sample had an initial microbial population of 1.1×10^7 cfu/g in the inoculum (*miso* sample). The total count of microorganisms increased during the first 10 days in all the products before becoming fairly stable until the fermentation was terminated after 60 days. Changes in the pattern of the microbial population of the products were quite similar. The initial count was found to be not very much different from the count at the end of 60 days of fermentation. The total microbial count for the products were in the range of $10^4 - 10^6$cfu/g. Similar trends were reported by Shieh *et al.*, (1982) in their study on the fermentation of peanut and soybean blends with *Aspergillus oryzae* molds.

The mold populations on the PCA declined rapidly during the early stages of fermentation and no colonies were detected in dilution of 10^{-3} after 40 days. The rapid fall in mold population may probably be due to the relatively high salt concentration (9%) used, (Hesseltine, 1983).

Various types of microbial colonies were observed on the plates as the fermentation progressed. Total microbial population was slightly higher in the cowpea-soybean sample as compared to the peanut-cowpea and peanut-soybean samples.

4.4.2 Microorganisms isolated during fermentation

Samples were periodically taken from the fermenting mixtures for the isolation and identification of the microorganisms involved at the various stages of fermentation. All isolates were gram-positive cocci. They were found primarily to belong to the *Micrococcaceae* and *Streptococcaceae* family of microorganisms.

By their characterization (Appendix 29 – 31), the main isolates were identified to be *Micrococcus*, *Streptococcus*, *Pediococcus* and *Staphylococcus* species. The major feature used to differentiate between the *Pediococcus* and *Streptococcus* species which were both catalase negative was the division of the *Pediococcus* cell along two planes to form tetrads while the *Streptococcus* species were either in singles or pairs. The *Micrococcus* and *Staphylococcus* were both catalase positive but the *Micrococcus* species could not ferment glucose to produce acid compared to the *Staphylococcus*. Additionally, the *Staphylococcus* species could not also ferment cellobiose.

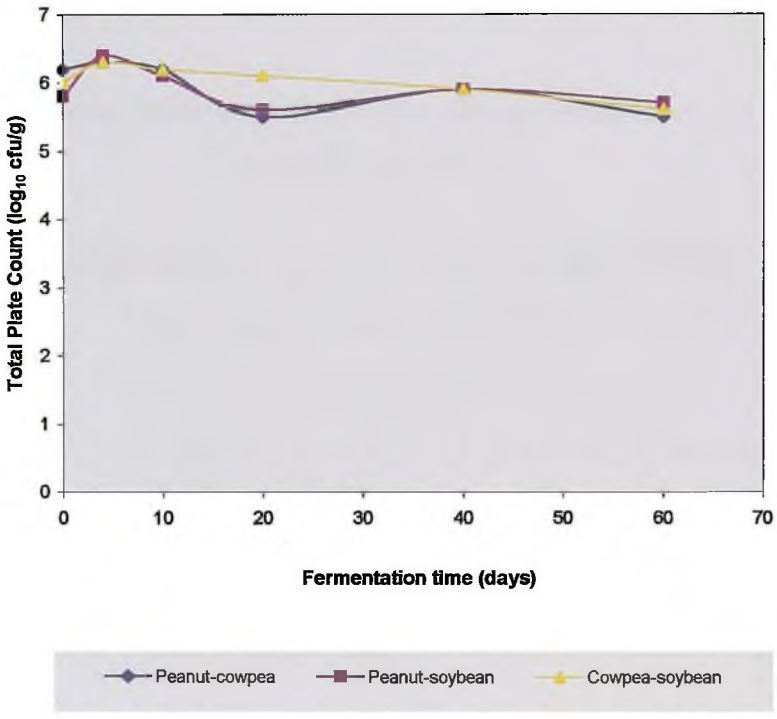


Fig. 4.7 Total microbial population of fermenting mixtures

Tables 4.2, 4.3 and 4.4 show the different microorganisms isolated from the products during the different stages of fermentation. The predominant species of microorganisms that appeared consistently throughout the 60 days of fermentation in all the three products were the *Micrococcus*, and *Pediococcus* species. These are mainly salt tolerant species and according to Ebine, (1986) *Pediococcus* species can survive and grow in brine solutions as high as 23%.

Table 4.2 Species of microorganisms isolated during fermentation (Peanut-cowpea product)

Fermentation time (days)	Microorganism isolated
0	*Micrococcus, Streptococcus, Staphylococcus, Pediococcus*
4	*Micrococcus, Streptococcus, Pediococcus*
10	*Micrococcus, Streptococcus, Staphylococcus, Pediococcus*
20	*Micrococcus, Pediococcus*
40	*Micrococcus, Pediococcus*
60	*Micrococcus, Pediococcus*

Table 4.3 Species of microorganisms isolated during fermentation (Peanut-soybean product)

Fermentation time (days)	Microorganisms isolated
0	*Micrococcus, Streptococcus, Pediococcus*
4	*Micrococcus, Streptococcus, Staphylococcus, Pediococcus*
10	*Micrococcus, Staphylococcus, Pediococcus*
20	*Micrococcus, Pediococcus*
40	*Micrococcus, Pediococcus*
60	*Micrococcus, Pediococcus*

Table 4.4 Species of microorganisms isolated during fermentation (Cowpea-soybean product)

Fermentation time (days)	Microorganism isolated
0	*Micrococcus, Streptococcus, Pediococcus, Staphylococcus*
4	*Micrococcus, Streptococcus, Pediococcus, Staphylococcus*
10	*Micrococcus, Streptococcus, Staphylococcus, Pediococcus*
20	*Micrococcus, Pediococcus*
40	*Micrococcus, Pediococcus*
60	*Micrococcus, Pediococcus*

The microbial profiles in all the three products were very similar. They belonged mostly to the lactic acid producing salt tolerant species with the exception of *Staphylococcus*. The *Staphylococcus* species occurred sporadically in the mixtures during the early stages of fermentation but disappeared from the mixture after 10 days of fermentation. The occurrence of *Staphylococcus* species in the fermenting mass may be attributed to contamination either from the raw materials or in the laboratory since they are not known organism to be involved in *miso* fermentation. The disappearance of the *Staphylococcus* species after 10 days of fermentation was considered to be advantageous since some species of *Staphylococcus* are known to be toxin producing. Though the microorganisms isolated from the three products were similar, they differed in count during the different stages of fermentation. It was generally a mix culture fermentation but involved only a few species that were able to survive throughout the period. The few species of microorganisms isolated from the fermenting mixtures may be because the legumes were virtually sterile after the boiling process before being mixed with the salted-*koji* and inoculum. According to Ebine, (1986) the high concentrations of salt used in formulating *miso* products inhibit the growth of weak salt-tolerant species such as *Streptococcus faecalis* and *Streptococcus faecium* that proliferate in the *koji*.

4.4.3 Changes in mold, yeast and LAB populations in the Peanut-cowpea sample

Changes in mold yeast and lactic acid bacteria population with fermentation time was monitored in the peanut-cowpea sample. The aim was to observe the population changes in the different groups of microorganisms as the fermentation progressed.

4.4.3.1 Mold population

The mold population decreased considerably from the initial value of 5.2 x 10^5 cfu/g to 6.0 x 10^4 cfu/g after 10 days, and to 4.2 x 10^2 cfu/g at the end of the fermentation process, (Fig 4.8). On the 60^{th} day of fermentation, the mold population declined to less than 4.0 cfu/g in dilution of 10^{-2}. Shieh *et al.* (1982) did not find any mold growth after 43 days of fermentation in a 10^{-1} dilution of a peanut and soybean substrate. This may be due to the higher concentration of salt (12 %) used as compared to 9 % in the present study. According to Hesseltine (1983) the drastic decrease in mold population with fermentation time could be mainly attributed to the high salt concentration used in the fermenting process and the nearly anaerobic conditions under which the fermentations were done. Chiou (1999) also suggested that the vegetative cells of microorganisms often show reduced heat resistance when exposed to NaCl.

The major role played by the mold is the production of amylolytic, proteolytic and lipolytic enzymes, which hydrolyze the substrates to lower molecular weight compounds. The yeast consequently acts on these low molecular weight compounds to produce flavorous compounds in the product (Sakurai, 1965 and Ebine, 1986). Amylolytic enzymes like amylase and maltase according to Shibasaki and Hesseltine (1962) breaks down starch mainly from the rice and cowpea to form dextrin maltose and glucose.

4.4.3.2 Yeast population

The initial population of yeast increased from about 2.7 x 10^4 cfu/g at the beginning of fermentation to a peak of 3.2 x 10^6 cfu/g after 20 days (Fig. 4.8). From then onwards, the total yeast population remained fairly constant to about the 40th day and then fell gradually till the fermentation process was terminated. The total yeast population after the 60 days of fermentation was about 9.0 x 10^3 cfu/g. The yeast isolates were identified to belong the species of *Saccharomyces rouxii,* (Appendix 32).

The fall in pH values as fermentation progressed was found to be suitable for the growth of yeast. This was because the yeast population was observed to initially increase as the pH of the mixtures dropped. Ebine, (1986) stated that *Saccharomyces rouxii* could grow in a medium with salt concentration as high as 18 % and a pH range of 4.0 – 5.0. According to Asao, (1961) the yeast acts on sugars like glucose and maltose to produce ethanol and phenolic compounds like 4-ethylguaicol, 4-ethylphenol and 2-phenylethanol which are important components of *miso* aroma. The ethanol produced during fermentation reacts with hydrolyzed free fatty acids to form ethyl esters that contribute a great deal to the *miso* flavour (Shieh *et al.*, 1982).

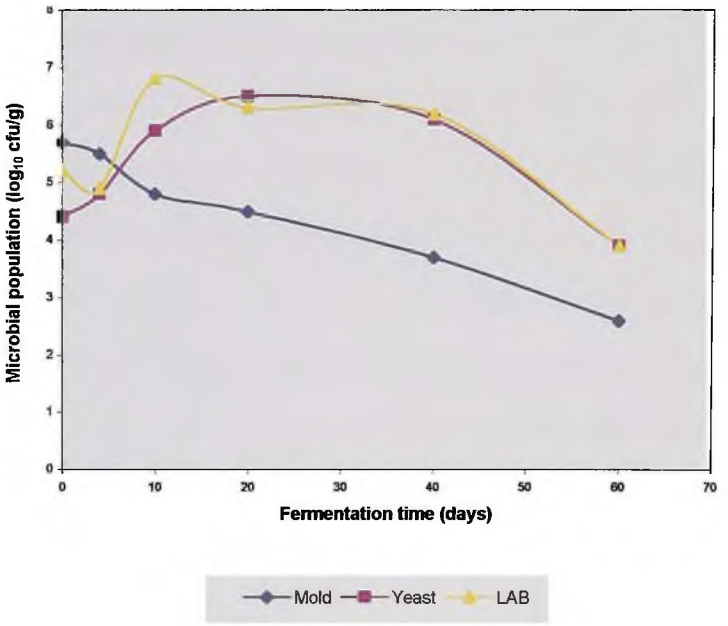


Fig. 4.8 Population of mold, yeast and LAB in peanut-cowpea sample

4.4.3.3 Lactic acid bacteria (LAB) population

Initial LAB population of 1.8 x 10^5 cfu/g decreased to 7.5 x 10^4 cfu/g during the first 4 days of fermentation before gradually increasing to a peak level of 6.3 x 10^6 cfu/g by the 10^{th} day (Fig. 4.8). From the 10^{th} day onwards the LAB population began to decrease gradually up to about the 40^{th} day and then rapidly towards the 60^{th} day of fermentation. Shieh *et al.*, (1982) did not find any particular pattern in the population of LAB during the fermentation of peanut and soybean *miso* samples. According to Hesseltine, (1983) the role of LAB in oriental fermentations is poorly known because of the lack of detail studies. Most investigations have been focused on the mold and yeast components rather than the bacterial flora. However, Ebine, (1986) stated that the major role of LAB in *miso* fermentation is to produce organic acids from carbohydrates, which lowers the pH of the mixture and this in turn accelerates the growth of the salt tolerant yeast. Esters are produced from the organic acids by reacting with ethyl and higher alcohols (Shibasaki and Hesseltine, 1962).

4.4 Proximate composition of samples

The proximate compositions of the dehydrated *miso*-like products are presented in Table 4.5. The products were dehydrated with the aim of prolonging the shelf-life and also, to make packaging easy.

Table 4.5 Proximate composition of samples (%)

Composition	Peanut-cowpea	Peanut-soybean	Cowpea-soybean
Moisture (%)	5.9 ±0.07	4.4 ± 0.07	5.6 ±0.07
Protein (%)	24.9 ±0.14	28.9 ± 0.07	32.6 ±0.10
Total lipids (%)	28.2 ±0.42	32.2 ± 0.35	10.5 ±0.57
Carbohydrates	17.23	13.7	25.1
Ash (%)	22.6 ±0.21	19.5 ± 0.05	25.0 ±0.29
Fiber (%)	1.17 ±0.07	1.3 ± 0.17	1.2 ±0.14

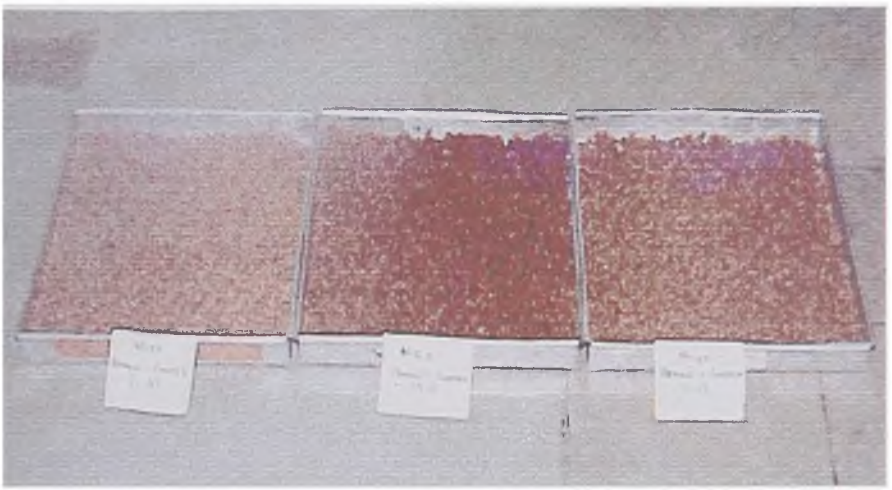
Moisture content of the products ranged from 4.4 to 5.9 % after drying at 40 °C for 24 hours. The cowpea-soybean sample contained the highest amount of crude protein followed by the peanut-soybean. Crude protein content in the range of 24.9 - 32.6 % observed in the samples was relatively high and it compared well with the range 15.5 - 28.6 % in soybean *miso* (Ebine, 1986). The peanut-cowpea and peanut-soybean samples were observed to contain high amounts of lipids as compared to the cowpea-soybean sample. As indicated in section 4.2.4, the lipid content of the products reflected the oil content of the component raw materials. Most of the lipids were derived from the peanuts. Total carbohydrates were estimated by subtraction to be in the range of 13.7 to 25.1 %. Some of the carbohydrates were in the form of reducing sugars. Reducing sugar concentrations were 10.5 %, 3.7 % and 11.9 %, respectively, for the peanut-cowpea, peanut-soybean and cowpea-soybean samples. According to Sakurai (1965), these sugars are utilized by yeast during the fermentation process to form alcohols. The low amount of reducing sugars in the peanut-soybean was most probably due to the low amount of carbohydrates in the peanut-soybean product.

Shieh *et al.*, (1982) observed the quantity of reducing sugars in *miso* samples made from peanut and soybean in the range of 4.8 - 9.5 % after 90 days of fermentation. Some portions of the carbahydrates were also converted by the LAB to form acids. Total acids formed in the products were determined to be 2.88 %, 2.38 % and 2.41 %, respectively, for the peanut-cowpea, peanut-soybean and cowpea-soybean products.

The three products contained appreciably high amounts of sulphated ash. These were in the range of 19.5 - 24.0 %. This compared well with the range of 10.9 - 26.9 % found in some varieties of *miso* (Ebine, 1986). Fiber content was observed to be quite low in all the products. The peanut-soybean sample had the highest amount, 1.3 % followed by 1.2 % in the cowpea-soybean and 1.17 % for the peanut-cowpea sample. The low fiber content observed may be due to the fact that the legumes were dehulled before used in the fermentation process. The fiber could also have been broken down into smaller units during the fermentation.

4.6 Total soluble solids

The amounts of soluble solids in the products were 33.1 %, 31.1 % and 26.1 % respectively, for the peanut-cowpea, peanut-soybean and cowpea-soybean samples. The relatively high amount of soluble solids in the samples was an indication that much proteins, lipids and carbohydrates had been degraded. These soluble nutrients are of great importance nutritionally, since they are highly digestible in the human gastro-intestinal tract.



Peanut:Cowpea (1:3) Peanut:Cowpea (3:1) Peanut:Cowpea

Plate 3: Pea-Cowpea *miso* samples with varying legume composition

4.7 Functional properties of the dehydrated *miso*

4.7.1 Water absorption

Water absorption capacities of the dehydrated *miso* products were in the range of 557 - 793 g/kg. Water absorption is largely accomplished by the protein bodies and starch granules, (Sefa-Dedeh and Stanley, 1979). The cowpea-soybean sample, which contained the highest amount of protein, showed the highest water absorbing capacity, followed by the peanut-soybean and the peanut-cowpea samples, respectively. The relatively high protein content of the samples may thus, be responsible for the high values observed. Water absorption capacity of the samples is an important functional property of proteins in viscous foods like soups where the water binding ability must be very suitable to make a soup with uniform consistency. The high water absorption capacity of the products goes to suggest that they could easily be made into soups, the traditional form in which *miso* is taken.

4.7.2 Fat absorption

Fat absorption capacity of the products as determined, fell in the range of 439.4 - 531.6g/kg for the three products. The highest amount of fat was absorbed by the peanut-soybean sample, which incidentally contained the highest amount of lipids. Fat absorption is basically, due to the physical entrapment of oil by protein. The hydrophobic portion of the protein, interact with the oil and physically entraps the oil. This allows for the penetration of oil into the granules. The above results infer that the samples could be easily mixed with oil in vegetable stews.

Table 4.6 Functional properties of samples

Functional property	Peanut-cowpea	Peanut-soybean	Cowpea-soybean
Water absorption(g/kg)	557 ±7.8	580 ±4.4	793 ±10.6
Fat absorption (g/kg)	439 ±32.7	532 ±33.1	485 ±32.7
Swelling capacity(%)	12.5 ± 0.38	29.4 ± 0.89	15.8 ± 0.73
Foaming ability (%)	5.5 ±0.35	2.6 ±0.14	1.5 ±0.42
Emulsifying capacity(%)	63.1 ±10.7	67.4 ± 4.6	57.8 ±8.6
Emulsion stability(%)	58.0 ±8.6	65.4 ± 7.3	60.0 ±5.5

4.7.3 Swelling capacity

The swelling capacity of the products, were in the range of 12.5 - 29.4%. The peanut-soybean, the sample with the highest lipid content was the product with the highest swelling capacity. This is an indication that lipids play a major role in swelling. Swelling is the expansion accompanying the uptake of water by cells. According to Sefa-Dedeh, (1979), during swelling the water molecules enter the cell granules and cause the solvation of the macromolecules and further occupy the capillary and intramolecular spaces of the molecules. The ability to imbibe water molecules may be influenced by the presence of hydrophilic polysaccharides (such as starch or amylose), lipids and salts. The extent of swelling varies with the protein sources, protein particle size, pH and ionic strength (Kinsella, 1976).

The rate of water up-take by the products is as shown in Figure 4.9. The peanut-soybean sample had the highest increase in volume, followed by the cowpea-soybean sample. The rate of swelling also differed for the different samples.

4.7.4 Foaming ability

No appreciable amounts of foam were formed in the products after whipping them in water for a minute. The foaming capacity of the products, were in the range of 1.5 - 5.5 %. The low foaming ability observed in the samples is desirable since *miso* is typically used as a soup base, which is ideally not required to foam. The little amount of foam formed was very stable in the peanut-cowpea sample but was reduced by 50% in the peanut-soybean and cowpea-soybean upon standing for an hour. Foam stability is known to be related to the amount of native proteins present in the sample (Lin *et al.*, 1974). After the fermentation and dehydration processes, the proteins were no longer in their native forms hence the observed low foaming ability associated with the samples.

Overall, functional properties such as high water absorption and swelling capacity, low foaming ability and the high fat absorption by the products go to suggest that they could be easily formulated into soups, the traditional form in which *miso* is used in the Oriental countries.

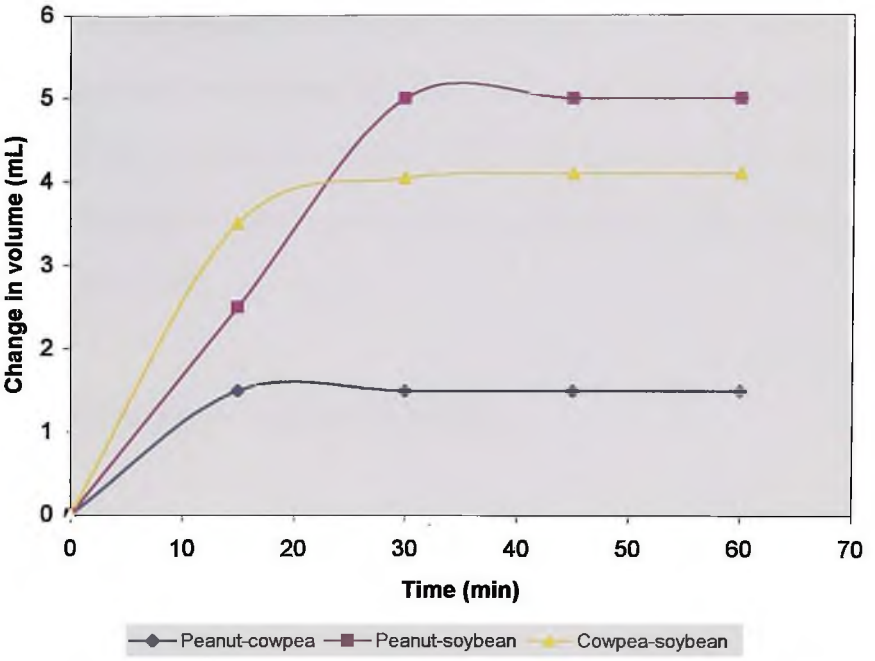


Fig. 4.9 Swelling rate of dehydrated samples

4.6.5 Emulsifying properties

The emulsifying capacities of the products ranged from 57.8 to 67.4 %. The emulsions were found to be quite stable after heating at 80 °C for a period of 30 minutes, (Table 4.6). This relatively high emulsifying capacity and emulsion stability is a desirable quality that could enhance the mixing of the products with an oily base food. Food emulsions are similar to classical emulsions consisting of fat droplets surrounded by a membrane matrix of protein (Kinsela, 1976). The emulsion capacity of proteins depend on the suitable balance between the hydrophilic and lipophilic characteristics, (Elizalde *et al.*, 1988).

4.8 Storage quality characteristics of products

4.8.1 Free Fatty Acids

Presented in Appendix 9 and as shown in Figure 4.10 is the data on the FFA determined in the products during storage. The FFA levels of the samples did not change much with treatment during the 12-week storage period. For the dehydrated samples, the levels of FFAs increased slightly from 0.18 to 0.31 %, and 0.12 to 0.19 % respectively, for the peanut-cowpea, and cowpea-soybean samples, but decreased from 0.29 to 0.20 % before rising up to 0.29 % in the peanut-soybean sample. For samples stored without any pre-storage treatment (untreated samples) the FFA levels increased slightly from 0.11 to 0.24 %, 0.19 to 0.22 % and 0.17 to 0.19 % respectively, for the peanut-cowpea, peanut-soybean and cowpea-soybean samples. In addition, for samples treated with 3% ethanol, the FFA values changed from 0.15 to

0.26 % and 0.19 to 0.22 % respectively, for the peanut-cowpea, peanut-soybean but remain stable at 0.17 % for the cowpea-soybean sample. The FFA values in the untreated samples were relatively lower as compared to the dehydrated samples. This may possibly be due to the active conversion of FFAs into esters as suggested by Shieh *et al.* (1982), due to the activities of the microbial population in the fresh samples. The levels of FFAs kept varying in the dehydrated and untreated samples for most of the storage period but remained fairly stable from the 4th week onwards in the ethanol treated sample.

Analysis of the data (Appendix 18) confirmed that the slight differences observed indicated that treatment of sample did not significantly ($p = 0.1346$) influence the FFA levels in the peanut-cowpea sample during the storage period. The FFA values however, increased significantly ($p = 0.008$) with storage time in the peanut-cowpea sample. For the peanut-soybean and cowpea-soybean samples, the FFA levels were neither significantly affected by treatment nor storage time, (Appendix 19 and 20). The above observations suggest that the method of pre-storage treatment may not significantly affect the storage stability of the *miso*-like products. The slight differences in the FFA levels may be due to the slight differences in the weight of the samples used for the determination.

The acid value is a measure of the extent to which the glycerides in an oil are hydrolyzed by lipase activity. According to Kirk and Sawyer (1991) the amount of FFAs formed in most oils must exceed 1.5% before acidity becomes noticeable on the palate. The relatively low amounts of FFA formed in the samples over the storage

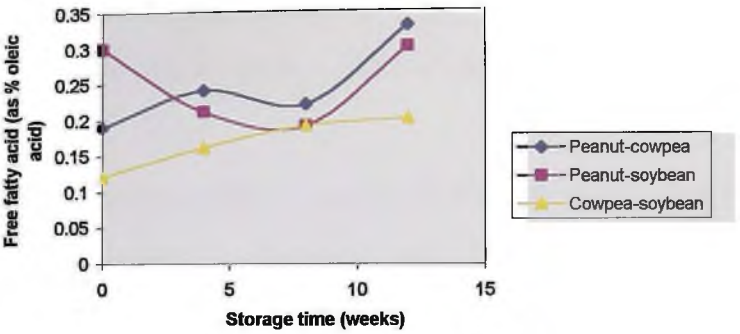


Fig. 4.10A Free fatty acid content of dehydrated samples

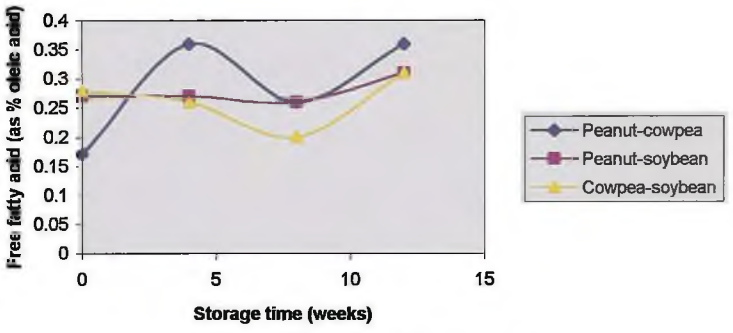


Fig. 4.10B Free fatty acid content of untreated samples

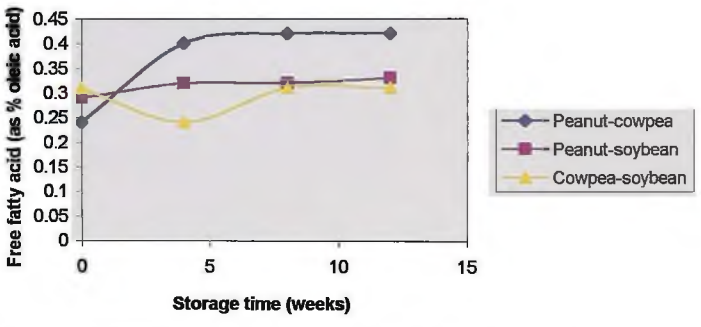


Fig. 4.10C Free fatty acid content of samples treated with ethanol

(on dry matter basis)

period (0.12 - 0.31) was an indication that the samples could store well at room temperature for a period of time, irrespective of the pre-storage treatment.

The transparent nature of the polythene sachets used for the storage may have contributed to the FFA formation in the products since exposure to light is known to hasten such free radical reactions. Although, peanuts and soybeans contain considerably high amounts of polyunsaturated fatty acids which could be easily broken down into FFA during fermentation, the low levels of FFA observed in the samples during storage period may give further support to the suggestion that FFA are actively converted into esters during fermentation. Mochizuki *et al.* (1972) observed that the decreases in FFA content of *miso* coincided with increases in FFA-ethyl ester content during fermentation. They also reported that glycerides are hydrolyzed to release FFA, which in turn continued to form the ethyl esters.

4.8.2 Peroxide value

Peroxide value of the products during storage, were as presented in Appendix 10 and as shown in Fig. 4.11. For the dehydrated products, the peroxide values of the peanut-cowpea sample increased gradually from 1.15 to 3.28 mEq/kg during the storage period. With the peanut-soybean and cowpea-soybean samples, the values increased during the first 8 weeks of storage but fell slightly by the 12th week. For the untreated sample, there was a general increase in the peroxide values of the peanut-cowpea and peanut-soybean samples with storage time but for the cowpea-soybean sample, there was rather a slight decrease from 3.83 to 3.47 mEq/kg during the same period. For the ethanol treated products there was a rapid increase from 1.5 to 2.94 mEq/kg for the first 4 weeks in the peanut-cowpea sample then gradually to a peak value of 3.49

mEq/kg after 12 weeks of storage. In the peanut-soybean sample the peroxide value increased only slightly from 1.33 to 2.03 mEq/kg over the same period. The cowpea-soybean sample unlike the other products showed an initial decrease from 2.85 to 2.19 mEq/kg in the peroxide values in the first 4 weeks before gradually rising to 2.73 mEq/kg after 12 weeks of storage.

Analysis of variance (Appendix 22) indicated that the amount of peroxides formed increased significantly ($p = 0.0008$) with storage time in the peanut-cowpea sample irrespective of the pre-storage treatment. These changes were mostly observed during the first 4 weeks of storage. With the peanut-soybean sample, neither the storage time nor treatment affected the peroxide values significantly, (Appendix 23). In the case of the cowpea-soybean sample, the peroxide values were significantly affected ($p= 0.0003$) by the pre-storage treatment, (Appendix 24). On conversion to dry matter, significant differences were observed in the peroxide values of the dehydrated, untreated and ethanol treated products during the storage period.

There were generally, increases in the peroxide levels of the products irrespective of the pre-storage treatment. The peroxide values which were below 4.0 mEq/kg, were far below the 20 – 40 mEq/kg range which rancidity begins to be noticeable in most oils (Kirk and Sawyer, 1991).

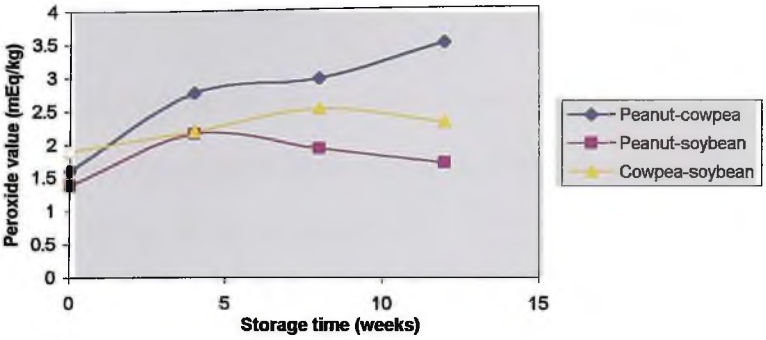


Fig.4.11A Peroxide value in dehydrated samples

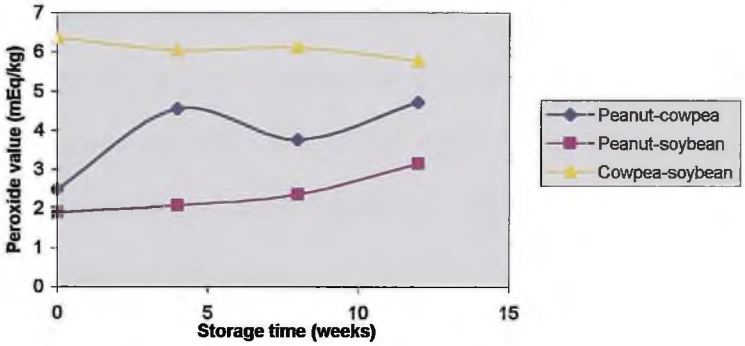


Fig. 4.11B Peroxide value in untreated samples

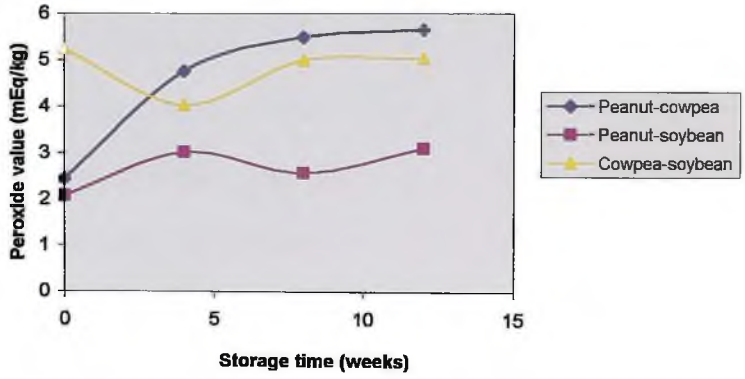


Fig. 4.11C Peroxide value in samples treated with ethanol

(on dry matter basis)

4.7.3 Thiobarbituric acid (as mg malonaldehyde/kg sample)

TBA measures the amount of red pigments formed in the reaction between 2-thiobarbituric acid and oxidized lipids as rancidity. TBA reactive substances were only determined for the peanut-cowpea samples.

There was a gradual fall in TBA values with storage time for all the samples. The TBA values were in the range of 0.008 - 0.02, 0.006 - 0.018 and 0.006 - 0.02 for the dehydrated, untreated and ethanol-treated samples respectively. Figure 4.12 shows the trend in the changes in the TBA values with storage time. From literature, refined oils in good condition should have TBA values in the range of 0.02 - 0.08. The low levels of TBA reactive substances found in the samples during storage indicated that there was not much lipid degradation in the samples and this could be attributed to a strong antioxidant activity generated during the fermentation process (Ebine, 1986). Traditionally, lipolysis has been attributed mainly to bacterial lipase activity (Roca and Incze, 1990). According to Gray (1978), lipid oxidation in fatty products is initiated when polyunsaturated fatty acids react with molecular oxygen via a free radical chain mechanism and form peroxides. The decreasing amount of TBA value with storage time further goes to buttress the earlier suggestion that the FFAs were being converted to ethyl-esters.

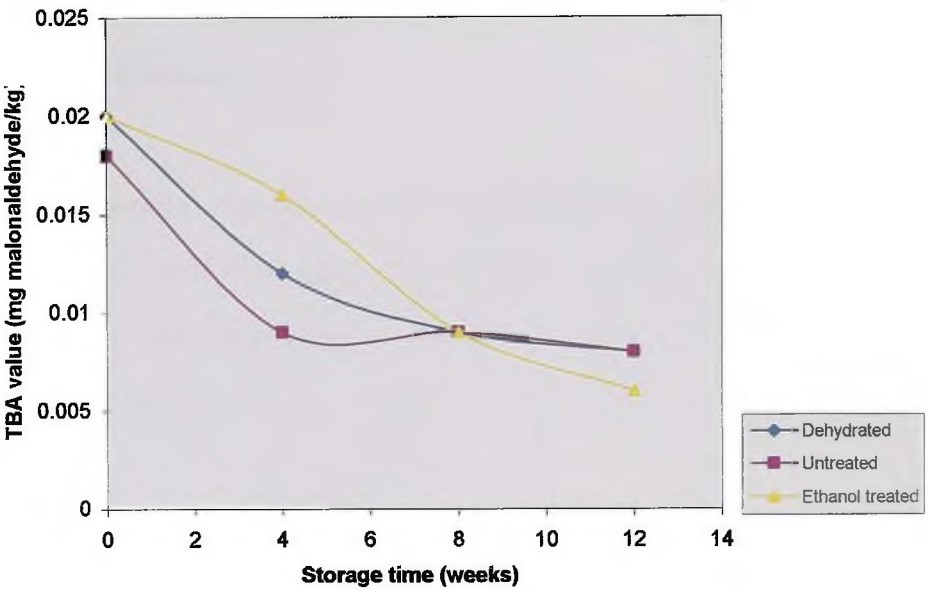


Fig. 4.12 Thiobarbituric acid value in peanut-cowpea sample

Factors that affect lipid oxidation include the composition of phospholipids, the amount of polyunsaturated fatty acids and the presence of a metal ion (Kanner *et al.*, 1988). Analysis of variance (Appendix 26) indicated that the rate of formation of TBA reactive substances in the peanut-cowpea product was significantly reduced ($p = 0.0006$) in all the samples after 4 weeks of storage irrespective of pre-storage treatment.

4.9 Sensory assessment

A sensory assessment was carried out to test the acceptability of the different *miso*-like products. A total of 30 participants were involved in the assessment, out of which 50% were males and 50% females. Table 4.7 shows the age distribution of persons who took part in the sensory assessment and Table 4.8 the occupational background.

Table 4.7 Age distribution of participants

Age group(yrs)	No.	%
< 20	6	20.0
20 - 30	8	26.7
31 - 40	8	26.7
> 40	8	26.7

Table 4.8 Professional background of participants

Profession	No.	%
Agriculturist	2	6.7
Health worker	7	23.3
Educationist	6	20.0
Accountant	4	13.3
Artisan	3	10.0
Students	6	20.0
Housewife	2	6.7

Table 4.9 Mean scores of acceptability test

Samples	Mean scores
Peanut-cowpea	4.87 ± 2.24
Peanut-soybean	5.07 ± 2.33
Cowpea-soybean	5.63 ± 1.99

From Table 4.9, the most acceptable product was the cowpea-soybean followed by the peanut-soybean. Thus, the peanut-cowpea was the least product accepted by the panel. Analysis of variance suggested that the legume combination did not exert a significant influence ($p = .1992$) on the product acceptability (Appendix 27). Product

acceptability on the other hand was however significantly influenced ($p = 0.0002$) by the panel. This shows that the peanut-cowpea was also acceptable to a good number of the panel.

The second section of the sensory analysis involved only the peanut-cowpea samples where, the effect of the legume proportion on the product preference was tested. The participants were asked to indicate their preference for the samples in a descending order. Table 4.10 shows the means of the preferential order given by the participants for the products.

Table 4.10 Mean scores of preferential assessment

Sample	Mean
peanut-cowpea(1:3)	1.80 ±0.76
Peanut-soybean(1:1)	2.33 ±0.84
Peanut-cowpea(3:1)	1.86 ±0.78

The most preferred among the peanut-cowpea combinations was the ratio of (1:3), followed by (3:1) and (1:1) combinations respectively.

Analysis of variance of the preferential (Appendix 28) test indicated that neither the panel nor the legume combination influence the product acceptability. This shows that the effect of the legume composition on product preference was insignificant. The

relatively low scores given in judging the acceptability of the products may be simply due to the unfamiliarity of the products to the panel.

4.10 General discussion

Raw materials were pretreated as for the traditional *miso* making process. The important treatments include cleaning, dehulling, soaking in water and cooking which was either by roasting or boiling. Dehulling was expected to remove the hard testa covering the grains and also to reduce the particle size of the legumes. Reduction in particle size of the grains was expected to hasten the growth of microorganisms and the rate of fermentation. The rates of dehulling vary for the different grain legumes. It was more difficult in the peanut since that was done manually compared to the cowpea and soybean samples, which were dehulled mechanically. The hulls contain some anti-nutritional factors as cited earlier in the Literature Review and their removal would help improve the nutritional quality of the products. Roasting came after the dehulling process and it was done to bring out flavour of the products. Production of flavour compounds through thermal hydrolysis has been observed in legumes (Woodroof, 1973). Though there was no literature available that indicates the dehulling and roasting of legumes in traditional *miso* preparation these processes were thought to be useful for the processing of local legumes into *miso*-like products. The main aim of boiling prior to the brine fermentation was to soften and precook the starch in the grains to facilitate microbial growth and substrate degradation. The periodic transfer of substrates into new pots carried out aeration of the fermenting

substrates. This was found necessary for the redistribution of the microorganisms and also to facilitate growth of the aerophylls.

In the traditional process of *miso* production, legumes and cereals are combined as indicated by Shieh et al. (1982). The combination of two legumes with cereals in this study was to experiment on the use of different legumes and also to obtain a substrate of composition considered feasible for the fermentation process. Local legumes vary considerably in their composition. Combining the cowpea with peanuts and soybean was to dilute the fat content of the products. It was also to modify the functionality of the products.

Profound changes were generally observed in the chemical characteristics of the substrates during the fermentation process. There was a decrease in pH in addition to increases in total acids, free amino nitrogen and total soluble nitrogen. These trends were an indication of the gradual breakdown of the substrates such as carbohydrates, proteins and lipids. The breakdown of the substrates resulted in the formation of more soluble components.

The observed growth of the culture and degradation of the substrates suggest that the inoculum developed for soybean *miso* is suitable for processing local grain legumes into *miso*-like products. According to Hesseltine (1983), the *Aspergillus* species used in *miso* production are non-toxin producers and therefore their growth on peanuts, which are associated with aflatoxins, will not cause any food safety problems. Another important observation was the elimination of the *Staphylococcus* species during the brine fermentation stage (Section 4.4.2). This was considered very important because

some *Staphylococcus* species are known toxin producers. The high concentration of salt used was generally expected to eliminate all pathogens that may have contaminated the fermenting mixtures.

The relatively high protein content and suitable functional properties were reflections of the characteristics of the raw materials used. These characteristics were good indications of the possible uses of the products.

Sensory evaluations also suggest that the products could be used in the formulation of local dishes.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The main task of this research work was to investigate the suitability of local grain legumes in the production of *miso*-like products. Legume combinations used to prepare the products were peanut-cowpea, peanut-soybean and cowpea-soybean. These were fermented at 35 °C for a period of 60 days. Data collected from the study shows that local grain legume like peanuts, cowpeas and soybeans could be suitable for processing into *miso*-like products.

The local legumes (peanuts, cowpeas and soybean) were able to withstand treatments like dehulling and cooking either by roasting or boiling. They were also able to support the inoculum used in the traditional *miso* production in the Orient. The relatively high levels of soluble solids in the products demonstrated the breakdown of macromolecules of the substrates into smaller units.

Combination of the legumes yielded products with different characteristics, which were dependent on the raw materials, used. From the three products made the most acceptable to the panel was the cowpea-soybean followed by the peanut-soybean and peanut-cowpea samples respectively. For the peanut-cowpea formulation the most preferred legume combination was the peanut : cowpea ratio of 1:3.

5.2 Recommendations

Since *miso* usage is not familiar locally, there is the need for further studies to improve the processing methods and come out with a product that can be easily assimilated into the local diet regimen. As a result, the following suggestions are being put forward for further consideration.

1. There should be a further study where the fermentation period could be extended for 90 days or more at 35 °C. This could degrade the substrates into more soluble forms and also improve the flavour.
2. Experimentation with other varieties of locally grown legumes in the *miso* process to select the most suitable variety.
3. High salt consumption is a major health concern for many people. For the *miso*-like products to be readily acceptable to many consumers there is the need to experiment with the use of relatively lower (4-6%) salt concentrations.
4. The use of local varieties of glutinous rice should be considered for the *koji* making process.
5. Promotional and educational campaigns should be mounted to create awareness of the importance of *miso*.
6. To consider using the consumer-type-sensory evaluation technique in further studies.

5.3 Problems encountered during the experimentation

The few problems encountered during the study include:

1. There was difficulty in obtaining information on *miso* processing technology.

2. English literature on miso making process was very scarce.

3. Media was not easily available for the microbiological analysis.

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APPENDIX

Appendix 1. Moisture content (%)

Fermentation time (days)	Peanut-cowpea (%)	Peanut-soybean (%)	Cowpea-soybean (%)
0	53.7 ± 0.49	46.7 ± 0.64	53.6 ± 0.49
4	53.8 ± 0.43	48.6 ± 0.07	57.6 ± 0.64
10	54.4 ±0.21	47.9 ± 2.05	57.9 ± 0.14
15	52.8 ± 0.85	48.6 ± 0.99	56.5 ± 0.28
20	53.6 ± 0.85	47.6 ± 0.07	54.1 ± 0.14
30	49.0 ± 1.48	42.7 ± 1.77	52.7 ± 0.35
40	43.0 ± 0.92	40.1 ± 2.40	47.1 ± 1.13
50	40.1 ±0.64	38.0 ±0.28	45.1 ±0.71
60	33.8 ±0.07	29.6 ±1.41	39.8 ±2.26

Appendix 2. Changes in pH values

Fermentation time (days)	Peanut-cowpea	Peanut-soybean	Cowpea-soybean
0	6.56	6.77	6.70
4	6.45	6.15	6.25
10	6.00	6.00	5.91
15	5.98	5.98	5.85
20	5.92	5.95	5.87
30	5.79	5.69	5.80
40	5.66	5.61	5.59
50	5.52	5.54	5.38
60	5.53	5.49	5.38

Appendix 3. Total Acid (as % w/w of lactic acid)

Fermentation time (days)	Peanut-cowpea %	Peanut-soybean %	Cowpea-soybean %
0	0.37 ± 0.03	0.40 ± 0.01	0.51 ± 0.01
4	0.88 ± 0.06	0.89 ± 0.02	0.72 ± 0.06
10	1.82 ± 0.03	2.06 ± 0.04	2.67 ± 0.00
15	1.71 ± 0.01	2.06 ± 0.04	2.60 ± 0.04
20	1.85 ± 0.18	2.13 ± 0.00	2.55 ± 0.03
30	1.84 ± 0.01	1.98 ± 0.02	2.49 ± 0.05
40	1.78 ± 0.03	1.95 ± 0.01	2.37 ± 0.02
50	1.89 ± 0.03	1.90 ± 0.02	2.43 ± 0.03
60	1.68 ± 0.01	1.72 ± 0.01	2.15 ± 0.01

Appendix 4. Total Free Amino Nitrogen (mg/g)

Fermentation time (days)	Peanut-cowpea (%)	Peanut-soybean (%)	Cowpea-soybean (%)
0	7.43 ± 0.47	7.43 ± 0.12	7.70 ± 0.36
4	14.23 ± 0.12	14.73 ± 0.23	15.0 ± 0.29
10	14.9 ± 0.20	15.07 ± 0.31	16.4 ± 0.20
15	14.73 ± 0.12	15.4 ± 0.20	16.33 ± 0.25
20	15.93 ± 0.25	15.07 ± 0.47	15.63 ± 0.23
30	17.33 ± 0.06	16.6 ± 0.20	16.83 ± 0.31
40	15.6 ± 0.20	17.03 ± 0.15	17.13 ± 0.12
50	14.3 ± 0.26	15.53 ± 0.40	16.07 ± 0.12
60	13.46 ± 0.12	14.37 ± 0.12	15.73 ± 0.12

Appendix 5 Total soluble Nitrogen (g/kg)

Fermentation time (days)	Peanut-cowpea %	Peanut-soybean %	Cowpea-soybean %
0	24.41 ± 0.30	32.74 ± 0.13	30.71 ± 0.16
4	27.17 ± 0.15	37.16 ± 0.55	33.96 ± 033
10	36.84 ± 0.31	43.95 ± 0.0	49.76 ± 0.17
20	42.35 ± 0.45	43.85 ± 0.21	51.10 ± 0.16
40	38.60 ± 2.73	43.74 ± 0.24	52.17 ± 0.0
60	39.88 ± 0.21	45.81 ± 0.10	47.26 ± 0.11

Appendix 6. Changes in protein content (%)

Fermentation time (days)	Peanut-cowpea %	Peanut-soybean %	Cowpea-soybean %
0	15.23 ± 0.15	20.36 ± 0.13	19.07 ± 0.16
4	16.99 ± 0.16	23.16 ± 0.29	21.11 ± 0.17
10	22.92 ± 0.16	27.45 ± 0.0	30.88 ± 0.0
20	26.5 ± 0.0	27.29 ± 0.27	32.02 ± 0.31
40	24.13 ±1.59	27.30 ± 0.12	32.61 ± 0.13
60	25.3 ± 0.96	27.97 ± 0.83	29.32 ± 0.11

Appendix 7. Total microbial population (cfu/g)

Fermentation time (Days)	Peanut-cowpea	Peanut-soybean	Cowpea-soybean
0	1.7×10^6	6.5×10^5	1.0×10^6
4	8.0×10^5	2.5×10^6	2.2×10^6
10	3.0×10^5	1.4×10^6	1.6×10^6
20	4.0×10^4	4.0×10^5	1.3×10^6
40	8.0×10^5	9.0×10^5	7.5×10^5
60	3.5×10^5	5.0×10^5	4.5×10^5

Appendix 8. Population of Mold, Yeast and Lactic acid bacteria (cfu/g)
Peanut-cowpea sample

Fermentation time (Days)	Mold	Yeast	Lactic acid bacteria
0	5.2×10^5	2.7×10^4	1.8×10^5
4	3.5×10^5	6.0×10^4	7.5×10^4
10	6.0×10^4	8.5×10^5	6.3×10^6
20	3.0×10^4	3.2×10^6	2.1×10^6
40	5.0×10^3	1.2×10^6	1.5×10^6
60	4.0×10^2	9.0×10^3	9.0×10^3

Appendix 9. Free Fatty Acids (% Oleic Acid) formed in samples during storage

(Dehydrated samples)

Time (wks)	Peanut-cowpea	Peanut-soybean	Cowpea-soybean
0	0.18 ± 0.015	0.29 ± 0.01	0.12 ± 0.018
4	0.23 ± 0.009	0.20 ± 0.004	0.15 ± 0.018
8	0.21 ± 0.008	0.18 ± 0.006	0.18 ± 0.015
12	0.31 ± 0.01	0.29 ± 0.007	0.19 ± 0.006

(Untreated samples)

Time (wks)	Peanut-cowpea	Peanut-soybean	Cowpea-soybean
0	0.11 ± 0.005	0.19 ± 0.014	0.17 ± 0.12
4	0.24 ± 0.005	0.19 ± 0.008	0.16 ± 0.007
8	0.17 ± 0.006	0.18 ± 0.012	0.12 ± 0.004
12	0.24 ± 0.01	0.22 ± 0.12	0.19 ± 0.008

(Ethanol treated samples)

Time (wks)	Peanut-cowpea	Peanut-soybean	Cowpea-soybean
0	0.15 ± 0.01	0.19 ± 0.01	0.17 ± 0.012
4	0.25 ± 0.015	0.21 ± 0.007	0.13 ± 0.005
8	0.26 ± 0.006	0.21 ± 0.012	0.17 ± 0.006
12	0.26 ± 0.02	0.22 ± 0.20	0.17 ± 0.017

(on "as is" basis)

Appendix 10. Peroxide values (mEq/kg) of samples during storage

(Dehydrated samples)

Time (wks)	Peanut-cowpea	Peanut-soybean	Cowpea-soybean
0	1.15 ± 0.14	1.32 ± 0.16	1.79 ± 0.09
4	2.60 ± 0.06	2.06 ± 0.04	2.06 ± 0.10
8	2.79 ± 0.36	1.83 ± 0.01	2.37 ± 0.16
12	3.28 ± 0.14	1.62 ± 0.07	2.16 ± 0.20

(Untreated samples)

Time (wks)	Peanut-cowpea	Peanut-soybean	Cowpea-soybean
0	1.64 ± 0.38	1.33 ± 0.09	3.83 ± 0.07
4	3.01 ± 0.10	1.46 ± 0.18	3.63 ± 0.13
8	2.49 ± 0.21	1.66 ± 0.13	3.67 ± 0.22
12	3.12 ± 0.06	2.21 ± 0.16	3.47 ± 0.13

(Ethanol treated samples)

Time (wks)	Peanut-cowpea	Peanut-soybean	Cowpea-soybean
0	1.50 ± 0.08	1.36 ± 0.11	2.85 ± 0.05
4	2.94 ± 0.24	1.98 ± 0.35	2.19 ± 0.10
8	3.39 ± 0.04	1.68 ± 0.45	2.71 ± 0.52
12	3.49 ± 0.05	2.03 ± 0.23	2.73 ± 0.04

(on "as is" basis)

Appendix 11. Thiobarbituric acids (mg malonaldehyde/kg sample) in the peanut-cowpea samples during storage

(dehydrated saples)

Time (wks)	TBA
0	0.020
4	0.012
8	0.009
12	0.008

(Untreated samples)

Time (wks)	TBA
0	0.018
4	0.009
8	0.009
12	0.006

(Ethanol treated samples)

Time (wks)	TBA
0	0.20
4	0.016
8	0.009
12	0.006

Appendix 12. ANOVA for moisture changes in the fermenting mixtures

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	1386.5104	10	138.65104	92.164	.0000
Ferm. Time	1073.6007	8	134.20009	89.205	.0000
Product	312.9096	2	156.45481	103.998	.0000
RESIDUAL	24.070370	16	1.5043981		
TOTAL (CORR)	1410.5807	26			

Appendix 13 ANOVA for pH changes in the fermenting mixtures

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	3.6828370	10	.3682837	58.838	.0000
Ferm. Time	3.6562519	8	.4570315	73.017	.0000
Product	.0265852	2	.0132926	2.124	.1521
RESIDUAL	.1001481	16	.0062593		
TOTAL (CORR.)	3.7829852	26			

Appendix 14. ANOVA for Total acid changes in the fermenting mixtures

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	11.574222	10	1.1574222	36.096	.0000
Ferm. Time	10.278600	8	1.2848250	40.069	.0000
Product	1.295622	2	.6478111	20.203	.0000
RESIDUAL	.5130444	16	.0320653		
TOTAL (CORR.)	12.087267	26			

Appendix 15 ANOVA for FAN changes in the fermenting mixtures

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	191.48651	10	19.148561	62.918	.0000
Ferm. Time	186.97974	8	23.372468	76.797	.0000
Product	4.50587	2	2.252937	7.403	.0053
RESIDUAL	4.869453	16	.30434412		
TOTAL (CORR)	196.35507	26			

Appendix 16 ANOVA for TSN changes in the fermenting mixtures

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	1034.3305	7	147.76150	20.608	.0000
Ferm. Time	764.2612	5	152.85224	21.318	.0000
Product	270.0693	2	135.03467	18.833	.0004
RESIDUAL	71.702256	10	7.1702256		
TOTAL (CORR.)	1106.0328	17			

Appendix 17. ANOVA for changes in protein of the fermenting mixtures

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	450.33281	7	64.333258	6.131	.0055
Ferm. Time	401.83383	5	80.366766	7.659	.0034
Product	48.49898	2	24.249489	2.311	.1496
RESIDUAL	104.93742	10	10.493742		
TOTAL (CORR.)	555.270223	17			

Appendix 18. ANOVA for Free Fatty Acid in the Peanut-cowpea sample

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	.0294417	5	.0058883	7.386	.0152
Treatment	.0045500	2	.0022750	2.854	.1346
Storetime	.0248917	3	.0082972	10.408	.0086
RESIDUAL	.0047833	6	7.9722E-004		
TOTAL (CORR)	.0342250	11			

Appendix 19. ANOVA for Free Fatty Acid in the Peanut-soybean sample

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	.0094750	5	.0018950	1.772	.2526
Treatment	.0043167	2	.0021583	2.018	.2137
Storetime	.0051583	3	.0017194	1.608	.2837
RESIDUAL	.0047833	6	7.9722E-004		
TOTAL (CORR.)	.0158917	11			

Appendix 20. ANOVA for Free Fatty Acid in the cowpea-soybean sample

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	.0011083	5	2.21667E-004	.222	.9399
Treatment	.0008167	2	4.08333E-004	.409	.6812
Storetime	.0002917	3	9.72222E-005	.097	.9586
RESIDUAL	.0059833	6	9.97222E-004		
TOTAL (CORR.)	.0070917	11			

Appendix 21 A. ANOVA for Free Fatty Acid in the dehydrated products

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	.0282333	5	.0056467	2.839	.1181
Storetime	.0121667	3	.0040556	2.039	.2100
Product	.0160667	2	.0080333	4.039	.0774
RESIDUAL	.0119333	6	.0019889		
TOTAL (CORR)	.0401667	11			

Appendix 21 B. ANOVA for Free Fatty Acid in the untreated products

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	.0180250	5	.0036050	1.330	.3649
Storetime	.0167583	3	.0055861	2.060	.2070
Products	.0012667	2	.0006333	.234	.7986
RESIDUAL	.0162667	6	.0027111		
TOTAL (CORR.)	.342917	11			

Appendix 21 C. ANOVA for Free Fatty Acid in the ethanol treated products

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	.0231417	5	.0046283	1.638	.2814
Storetime	.0104250	3	.0034750	1.230	.3778
Products	.0127167	2	.0063583	2.251	.1865
RESIDUAL	.0169500	6	.0028250		
TOTAL (CORR.)	.0400917	11			

Appendix 22. ANOVA for Peroxide value in the Peanut-cowpea sample

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	5.3796667	5	1.0759333	16.352	.0019
Treatment	.2024667	2	.1012333	1.539	.2888
Storetime	5.1772000	3	1.7257333	26.227	.0008
RESIDUAL	.3948000	6	.0658000		
TOTAL (CORR)	5.7744667	11			

Appendix 23. ANOVA for Peroxide value in the Peanut-soybean sample

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	.6610167	5	.1322033	2.012	.2097
Treatment	.0191167	2	.0095583	.146	.8675
Storetime	.6419000	3	.2139667	3.257	.1017
RESIDUAL	.3941500	6	.0656917		
TOTAL (CORR)	1.0551667	11			

Appendix 24. ANOVA for Peroxide value in the cowpea-soybean sample

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	5.1375667	5	1.0275133	16.835	.0018
Treatment	5.0060667	2	2.5030333	41.011	.0003
Storetime	.1315000	3	.0438333	.718	.5764
RESIDUAL	.3662000	6	.0610333		
TOTAL (CORR)	5.5037667	11			

Appendix 25 A. ANOVA for Peroxide value in the dehydrated sample

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	3.1946083	5	.6389217	4.278	.0527
Storetime	1.50915833	3	.5030528	3.369	.0959
Product	1.6854500	2	.8427250	5.643	.0418
RESIDUAL	.8960167	6	.1493361		
TOTAL (CORR)	4.0906250	11			

Appendix 25 B. ANOVA for Peroxide value in the untreated sample

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	31.2112175	5	6.242243	14.725	.0026
Storetime	1.9810003	3	.660333	1.558	.2943
Products	29.230217	2	14.615108	34.477	.0005
RESIDUAL	2.5434500	6	.4239083		
TOTAL (CORR.)	33.754667	11			

Appendix 25 C. ANOVA for Peroxide value in the ethanol treated sample

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	14.080292	5	2.8160583	3.354	.0864
Storetime	3.106092	3	1.0353639	1.233	.3768
Products	10.974200	2	5.4871000	6.536	.0311
RESIDUAL	5.0371333	6	.8395222		
TOTAL (CORR.)	19.117425	11			

Appendix 26. ANOVA for Thiobarbituric acid value in the cowpea-soybean sample

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	2.84833E-004	5	5.69667E-005	18.149	.0015
Treatment	1.11667E-005	2	5.58333E-006	1.779	.2474
Storetime	2.73667E-005	3	9.12222E-005	29.062	.0006
RESIDUAL	1.88333E-005	6	3.13889E-006		
TOTAL (CORR)	3.03667E-004	11			

Appendix 27. ANOVA for Acceptability test of the products

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	261.94444	31	8.4498208	2.955	.0002
Treatment	9.48889	2	4.7444444	1.659	.1992
Storetime	252.45556	29	8.705364	3.044	.0002
RESIDUAL	165.84444	58	2.8593870		
TOTAL (CORR)	427.78889	89			

Appendix 28 ANOVA for Preference test for the different proportions of the peanut-cowpea sample

Source of variation	SS	d.f.	MS	F-ratio	Sig. level
MAIN EFFECTS	5.0666667	31	.1634409	.179	1.0000
Treatment	5.0666667	2	2.5333333	2.675	.0774
Storetime	.0000000	29	.0000000	.000	1.0000
RESIDUAL	54.93333	58	.9471264		
TOTAL CORR)	60.000000	89			

Appendix 29a . Morphological and biochemical properties of bacteria from the peanut-cowpea sample during fermentation

Day of fermentation

	Day = 0					Day = 4					Day = 10				
Characteristics	**P1**	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**	**P9**	**P10**	**P11**	**P12**	**P13**	**P14**	**P15**
Shape	CP	CL	CP	CT	CP	CP	CP	CS	CT	CP	CL	CS	CT	CP	CP
Gram Stain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+w	+	-	-	+	-	+	-	+	+	+w	-	-	-
Pigment	cw	gy	cr	cw	cw	cr	cr	cw	cw	cr	gy	cw	cw	cr	cw
Motile	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	-	+	-	+	+	-	+	-	+	-	+	-	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	-	+	+	+	+	+	+	+	-	-	+	+	+	+w
Mannitol	-	-	+	+w	-	+	-	+	+	+	-	+	-	-	+w
Cellobiose	-	-	-	+	+	-	-	-	+	-	-	+w	+	+	+
Maltose	+	+	+	+w	+	+	+	+	+	+	+	+	+	+	+
Xylose	+	-	+	-	-	+	-	+	+	+	-	-	+	-	-
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	-	+	+	-	-	+	-	+	-	+	+	+	-	-	-

Legends : C = coccus pairs; CL = coccus clusters; CT = coccus tetrads; CS = coccus singles; cw = cream-white; cr = cream; gy = golden-yellow ; y = yellow; + = positive reaction; - = negative reaction; +w = weak reaction, P = strain number

Genus : Streptococcus spp (P5, P7 and P14)
Micrococcus spp (P1, PP3, P6, P8 and P12)
Pediococcus spp (P4, P9, P13 and P15)
Staphylococcus spp (P2 and P11)

Appendix 29b. Morphological and biochemical properties of bacteria from the peanut-cowpea sample during fermentation

Day of fermentation

	Day = 20					Day = 40					Day = 60				
Characteristics	**P18**	**P19**	**P20**	**P21**	**P22**	**P26**	**P27**	**P28**	**P29**		**P30**	**P31**	**P32**	**P33**	**P34**
Shape	CT	CS	CP	CT	CT	CP	CT	CT	CT		CP	CT	CT	CP	CT
Gram Stain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Catalase	-	+	+	-	-	+	-	+w	-		+	-	-	+	-
Pigment	cr	cw	cw	y	cw	cw	cw	cw	y		cr	cw	cw	cr	cw
Motile	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Glucose	+	-	-	+	+	-	+	+	+	-	+	+	-	+	
Sucrose	+	+	+	+	+	+	+	+	+		+	+	+	+	+
Lactose	-	+	+	+	+	+	+	+	+		-	+	+	+	+
Mannitol	-	-	+	-	+	-	-	+	-		+	-	+	+	-
Cellobiose	-	-	+	+	+	-	+	-	+		-	+	+	-	-
Maltose	-	+	+	+	+	+	+	+	+	+	-	+	-	+	
Xylose	-	+	+	-	+	+	-	+	-		+	-	+	-	-
Mannose	+	+	-	+	+	+	+	+	+	-	+	+	+	+	
Sorbitol	-	+	+	-	-	+	-	-	-	+	-	-	+	-	

Legends : C = coccus pairs; CL = coccus clusters; CT = coccus tetrads; CS = coccus singles; cw = cream-white; cr = cream; y = yellow; + = positive reaction; - = negative reaction; +w = weak reaction, strain number

Genus : Micrococcus spp (P19, P20, P26, P30, P33, and P35)
Pediococcus spp (P18, P21, P22, P27, P28, P29, P32 and P34)

Appendix 30a. Morphological and biochemical properties of bacteria from the peanut-soybean sample during fermentation

Day of fermentation

	Day = 0					Day = 4					Day = 10			
Characteristics	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**	**S13**	**S14**
Shape	CT	CP	CT	CP	CP	CP	CP	CT	CS	CL	CL	CT	CP	CP
Gram Stain	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	-	+	-	-	-	-	+	-	+	+	+	-	+w	-
Pigment	cw	gy	cr	cw	cw	cr	cr	cw	cw	cr	gy	cw	cw	cr
Motile	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	-	+	+	+	+	+	-	+	-	+	+	+	-	-
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	-	+	-	+	+	+	-	-	+	+	+
Mannitol	-	-	-	-	-	-	+	-	-	+	+	-	-	-
Cellobiose	-	-	+	+	+	+	+	-	+	+	-	+	-	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Xylose	-	+	-	-	-	-	+	-	+	-	-	-	+	-
Mannose	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Sorbitol	-	+	-	-	+	-	+	-	-	+	+	-	+	-

Legends : C = coccus pairs; CL = coccus clusters; CT = coccus tetrads; CS = coccus singles; cw = cream-white; cr = cream; gy = golden-yellow; y = yellow; + = positive reaction; - = negative reaction; +w = weak reaction, S = strain number

Genus : Streptococcus spp (S4, S5 and S6)
Micrococcus spp (S2, S7, S9 and s13)
Pediococcus spp (S1, S3, S8, S12 and S14)
Staphylococcus spp (S10 and S11)

Appendix 30b. Morphological and biochemical properties of bacteria from the peanut-soybean sample during fermentation

Day of fermentation

	Day = 20					Day = 40					Day = 60			
Characteristics	**S15**	**S16**	**S17**	**S18**	**S20**	**S22**	**S23**	**S24**	**S25**	**S26**	**S28**	**S29**	**S30**	**S31**
Shape	CT	CT	CP	CS	CP	CT	CP	CT	CP	CT	CT	CP	CT	CP
Gram Stain	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	-	-	+	-	+	-	+	-	+	-	-	+w	-	+
Pigment	cr	cw	cw	cr	cw	cr	cw	cr	cw	cr	y	cw	cw	cr
Motile	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	-	+	-	+	+	-	+	-	+	-	+	-	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	-	+	+	+	+	+	+	+	-	-	+	+	+
Mannitol	-	-	+	+w	-	+	-	+	+	+	-	+	-	-
Cellobiose	+	-	-	+	-	+	-	-	-	+	-	+w	-	-
Maltose	+	+	+	+w	+	+	+	+	+	+	+	+	+	+
Xylose	-	+	+	-	-	+	-	-	+	-	-	+	+	-
Mannose	+	+	-	+	+	+w	+	+	-	+	-	+	+	+
Sorbitol	-	-	+	-	+	-	+	-	+	-	-	+	-	+

Legends : C = coccus pairs; CL = coccus clusters; CT = coccus tetrads; CS = coccus singles; cw = cream-white; cr = cream; gy = golden-yellow; y = yellow; + = positive reaction; - = negative reaction; +w = weak reaction

Genus : Micrococcus spp (S17, S20, S23, S25, S29 and S31)
Pediococcus spp (S15, S16, S18, S22, S24, S26, S28 and S30)

Appendix 31a. Morphological and biochemical properties of bacteria from the cowpea-soybean sample during fermentation

Day of fermentation

	Day = 0					Day = 4				Day = 10			
Characteristics	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**	**C12**	**C13**
Shape	CL	CP	CT	CP	CP	CP	CP	CP	CT	CT	CP	CL	CP
Gram Stain	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	-	-	+	-	+	+	-	-	-	+w	+	-
Pigment	gy	cw	cw	cr	cw	cr	cw	cw	cw	cw	cw	y	cr
Motile	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	-	+	-	-	+	+	+	-	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	-	+	+	+	-	+	+	-	-	+	+	+
Mannitol	-	-	-	+w	-	+	+	-	+	-	+	+	-
Cellobiose	-	+	+	-	+	-	-	+	-	+	-	-	+
Maltose	+	+	+	+w	+	+	+	+	+w	+	+w	+	+
Xylose	+	-	-	+	-	+	+	+	+	-	+	+	-
Mannose	+	+	+	+	-	-	+	+	+	+	+	+	-
Sorbitol	+	+	-	-	+	+	+	-	-	-	+	+	-

Legends : C = coccus pairs; CL = coccus clusters; CT = coccus tetrads; CS = coccus singles; cw = cream-white; cr = cream; gy = golden-yellow ; y = yellow; + = positive reaction; - = negative reaction; +w = weak reaction; C = strain number

Genus : Streptococcus spp (C2, C5, C8 and C13)
Micrococcus spp (C4, C6, C7 and C11)
Pediococcus spp (C3, C9 and C10)
Staphylococcus spp (C1 and C12)

Appendix 31b. Morphological and biochemical properties of bacteria from the cowpea-soybean sample during fermentation

Day of fermentation

	Day = 20					Day = 40			Day = 60				
Characteristics	**C15**	**C16**	**C17**	**C18**	**C19**	**C22**	**C23**	**C24**	**C26**	**C27**	**C28**	**C29**	**C30**
Shape	CT	CP	CP	CT	CT	CT	CT	CS	CT	CT	CP	CP	CT
Gram Stain	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	-	+	+	-	-	-	-	+	-	-	+	+	-
Pigment	cw	cw	y	cr	cw	cw	cr	cw	cw	cr	cw	y	cw
Motile	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	-	-	+	+	+	+	-	+	+	-	-	+
Sucrose	+	+	-	+	+	+	+	+	+	+	+	-	+
Lactose	-	+	+	+	+	-	+	+	-	+	+	+	-
Mannitol	-	-	-	+w	-	-	-	+w	-	+	+	-	+w
Cellobiose	+	-	-	-	+	+	-	-	+	-	-	-	+
Maltose	+	+	-	+w	+	+	+	+	+	+	+	-	+
Xylose	+	-	+	-	-	-	-	+	-	-	+	+	-
Mannose	+	+	-	+	+	+	-	+	-	+	+	-	+
Sorbitol	-	+	+	-	-	-	-	+	-	-	+	+	-

Legends : C = coccus pairs; CL = coccus clusters; CT = coccus tetrads; CS = coccus singles; cw = cream-white; cr = cream; y = yellow; + = positive reaction; - = negative reaction; +w = weak reaction; C = strain number

Genus : Micrococcus spp (C16, C17, C24, C28 and C29)
Pediococcus spp (C15, C18, C19, C22, C23, C26, C27 and C30)

Appendix 32

Biochemical properties of Yeast colonies

	Isolated Yeast Colonies		
Characteristics	**Y1**	**Y2**	**Y3**
Growth	+	+	+
Glucose	+	+	+w
Maltose	+	+	+
Lactose		-	
Galactose	-	-	
Sucrose	-		+w
Inulin	+	-	-

Appendix 33

ACCEPTABILITY TEST FOR *MISO*-LIKE PRODUCTS (A)

Date: ..../..../.... Sex: 1. Male 2. Female

Age group(yrs): 1. Less than 20, 2. (20 - 30), 3. (30 - 40), 4. Above 40.

The samples presented to you are fermented leguminous products that are typically used as a soup base. They could also be used to flavour vegetable stews. Kindly taste these samples and indicate your likeness from left to right by circling a corresponding number on the scale provided (0 - 9). An indication of zero (0)means you don't like the product at all.

Samples	Level of likeness	
PC	Don't like it	0----1----2----3----4----5----6----7----8----9 Like very much
PS	Don't like it	0----1----2----3----4----5----6----7----8----9 Like very much
CS	Don't like it	0----1----2----3----4----5----6----7----8----9 Like very much

PREFERENCE TEST FOR PEANUT-COWPEA SAMPLES (B)

These samples are similar to the ones presented in section 'A' above. Kindly taste the samples and indicate your preference by circling the corresponding number.

For example:
1. Most preferred
2. Next preferred
3. Least preferred

Samples:
P (1:3)
P (1:1)
P (3:1)