

**SOME MICROBIOLOGICAL AND BIOCHEMICAL STUDIES ON  
THE FERMENTATION OF TWO TRADITIONAL CONDIMENTS,  
'AWAZE' AND 'DATTA**



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**by  
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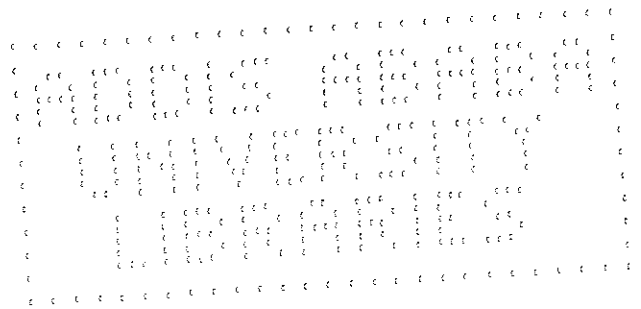
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## Abstract

The microbial dynamics , pH and titratable acidity together with some biochemical changes associated with the fermentation of two Ethiopian condiments, '*awaze*' and '*datta*' were investigated following the traditional methods of preparation. The two condiments were prepared in the laboratory, from a variety of vegetable and spice mixtures. The fermentation of both condiments is carbohydrate fermentation carried out by lactic acid bacteria with a progressive increase in titratable acidity and decline in pH. Other microorganisms isolated did not appear to play a major role in the process of fermentation, although yeasts appeared in the latter stage of '*awaze*' fermentation. Lactic acid bacteria, which kept on increasing throughout the fermentation, ranged from  $10^6$  –  $10^{11}$  cfu/g for '*awaze*' and from  $10^4$ – $10^9$  for '*datta*'. Enterobacteriaceae and coliforms, which were isolated at the initial stages of '*datta*' fermentation, were inhibited at 32 h. by the antimicrobial activity of the dominating lactic acid bacteria. Reducing sugar changes, detected by the DNS-colorimetric method, was very significant in '*awaze*' than '*datta*', in which there was an initial increase, and a decline afterwards due to the utilization of sugar by the proliferating lactics. The changes associated with the protein content of the two condiments, as detected by the formol titration method, were very little.

## 1. INTRODUCTION

Fermentation, an important aspect of food microbiology, was discovered many centuries ago as means of processing and preserving food (Steinkraus,1983a). Its discovery goes even before written records and therefore the process is usually referred to as traditional food fermentation (Hesseltine, 1983a). Many such processes, under the blanket term “fermentation”, have been the usual duties of ancient people for the past thousands of years (Coultate, 1995). In all these, microbial activity is utilized to produce the respective food items. Cheese making and pickling, for instance are both bio-deteriorations where microbial activities convert the substrate to a more unconvertible product (Coultate, 1995). Many other food fermentation processes yielding dairy products, sausages and sauerkraut have also been well investigated and documented (Hesseltine and Wang,1980).

Although the history of most fermented foods extends back into antiquity and no precise time can be established for their origin, the fact that fermentation occurred was totally beyond the recognition of the ancient people (Pederson,1979). When vegetables, for example, flavored with salt brine were packed tightly in a vessel, they changed in character but remained appetizing and nutritious (Pederson, 1979), but people were not aware that microbial activity and fermentations were responsible for the change until Louis Pasteur

ingeniously proved that micro-organisms are involved in the fermentation of various foods.

In many parts of the world, particularly in developing countries, fermented food products constitute the major staple foods (Neelam and Chauhan, 1990; Antony and Chandra, 1997). Some fermented foods produce strong flavor such that the product is not consumed alone, but is added as a condiment to make the food more tasty and enjoyable (Hesseltine 1983a). There are many traditional condiments in different parts of the world produced by microbial fermentations. Such traditional condiments as 'daddawa'(Ogbadu *et al.*1990), and 'iru' (Odunfa and Oyewole, 1986) of Nigeria; Soy Sauce of Japan (Luh,1995) and "Sufu' and "Miso" of Asia (Hesseltine and Wang,1980) and Siljo of Ethiopia (Tetemke and Mogessie,1995) are used as taste enhancers in many traditional dishes. The microbiological and biochemical properties of the fermentations of these condiments have also been studied by many workers.

In Ethiopia two traditional condiments are '*awaze*' and '*datta*' and are consumed with other items on the basis of their desirable aromas and flavours. Both condiments result from the microbial fermentations of vegetable-spice mixtures. The major substrates in '*awaze*' are red sweet pepper (*Capsicum annum*) garlic (*Allium-ursinum* and ginger (*Zingiber officinale*) with which some proportions of different spices are added . The major substrate in the

making of 'datta' is the small Chili pepper (*Capsicum frutescence*) at its green stage.

'Awaze', as a condiment, is commonly known in the North and Central Ethiopia and is often consumed with sliced raw meat (Kurt), and 'Injera' (traditional pancakes). It is also added to enhance the flavor of 'Shiro wat' a powdered bean sauce (Belaynesh, 1978 and Tsigie, 1990). 'Datta' is used as a condiment and a side dish mainly in the southern parts of the country such as Awassa., Arba Minch and Welayita areas, and in some parts of Wollo, and Bati areas where it is consumed with bread, sliced raw meat, and 'Kitta', traditional unleavened bread (personal communication)

Research made on the fermentation of many plant materials revealed that most vegetable fermentations are initiated by *Leuconostoc mesenteriodes* (Berhanu Abegaz, 1987) ,*Lactobacillus plantarum* and other *Lactobacillus* spp. have been isolated at some stages in the fermentation of vegetables (Pederson, 1979). Palumbo *et.al.* (1974) showed that spices often contain large numberof bacteria such as *Bacillus* spp. and molds. The majority of these fermentations are accompanied by certain biochemical changes of nutritional importance .

While the microbiology and biochemical properties of several other fermented foods have been studied, There are no reports on the fermentation of these two

indigenous Ethiopian condiments. Thus the present study will be undertaken to investigate certain microbiological and biochemical changes during the fermentation of '*awaze*' and '*datta*'.

## 2. LITERATURE REVIEW

### *2.1. Fermentation in Food Preservation*

Human foods are naturally characterized by having moderate pH values, mild temperatures and a wide range of oxygen tension (Coultrate, 1995). This makes the majority of foods suitable to harbour obligate anaerobes, obligate aerobes as well as fastidious types suggesting that many food items are inevitably ideal carriers of pathogenic bacterial species such as *Salmonella*. They are also good substrates for the growth of bacteria capable of secreting toxins such as *Clostridium botulinum*, *Bacillus cereus* and *Staphylococcus aureus* (Jay,1996).

According to the National Research Council (1983), developing countries face losses of food due to contamination, pests and microbial spoilage during storage. Such Methods of preserving food like canning, freezing and dehydration with heat are so expensive to the developing world that an alternative method of increasing the food supply should be looked for (Steinkraus, 1983b).

Among the most crucial aspects of food science, food safety is nowadays gaining importance with the public at large and by microbiologists in particular. This is achieved by preservation using fermentation (Hesseltine,1983a; Jay,1996).

Food spoilage is becoming the most serious economic problem in the food processing industry (Brock and Madigan, 1991). Most food spoilage bacteria do not, however, grow at pH values below 5. Hence one aspect of food preservation is making conditions suitable that allow acidity to develop directly in the food through microbial action to get fermented food. Pickling for instance is a traditional way of preserving food which makes use of the microbial conversion of sugars into lactic acid by the activity of lactic acid bacteria. These bacteria create high acidic environment and inhibit the growth of other food spoilage microorganisms. Preservation of food is not merely due to the acidity produced by lactic acid bacteria but also due to the production of bacteriocins, small ribosomally synthesized antimicrobial peptides (Vincent *et.al.*, 1998) which are added to several foods as means of preservation. Prevention of spoilage in many cereal African fermented foods is achieved by the activity of these bacteriocin-producing lactic acid bacteria (Holzapfel, 1995).

Several vegetables can be made to acquire a longer shelf life by using this biological method of preserving food by the action of bacteria and yeasts that are normal flora of the fresh ingredient. In many cases the vegetables are sorted, washed and then immersed in brine or dry salt under shade in the open air (Agricole, 1996). The lactic acid bacteria make spontaneous fermentation provided there is ambient temperature and correct concentration of sodium

chloride. Pawpaw (*Carica papaya*), for instance, can be kept in brine for long by means of lactic fermentation and the product becomes not only delicious but also retains its vitamins and nutrients (Agricole,1996).

## **2.2. Health Benefits of Fermented Foods**

### **2.2.1. Lactic Antagonism**

The inhibition or killing by lactic acid bacteria of food poisoning or food spoilage organisms is referred to as lactic antagonism (Jay, 1996). It may be the result of many factors such as high acidity, production of bacteriocins, H<sub>2</sub>O<sub>2</sub>, diacetyl, and/or nutrient depletion. Lactic antagonism produced by the activity of lactic/acetic acid bacteria is very important against certain pathogens (Miller and Martin, 1996). These workers experimentally observed that when an initial inoculum of  $5 \times 10^6$  *Salmonella enteritidis* and *S. typhimurium* were inoculated into an oil and vinegar based salad dressing, no count of *S. enteritidis* and *S. typhimurium* could be detected after 5 and 10 minutes, respectively, signifying the importance of lactic antagonism.

Many bacteriocins are also involved in such lactic antagonisms and prevent growth of food-borne pathogens (Olasupo *et, al.* 1995; Kaiser and Montiville, 1996). The largest number of these bacteriocins are produced by lactobacilli followed by lactococci (Nettles and Barefoot, 1993)

### **2.2.2. Fermented Foods Against Infection and Other Diseases**

Diarrhoeal infection is causing an increased child mortality and morbidity in many developing countries. Such causative agents as enterotoxigenic *E. coli* (ETEC), *Shigella*, *Salmonella* and *Campylobacter* have been reported elsewhere (Gross, 1983). One of the major routes of transmission of these enteropathogens is either food or water. Child diarrhoea is mostly associated with contaminated dairy products. However, cereals being the principal source of food for most developing nations, more often than not origins of diarrhoea in these countries are mainly cereal-based diets (Lorri and Svanberg, 1994). The problems caused by diarrhoeal pathogens can effectively be reduced or eliminated by lactic acid fermentation of cereal-based foods. Gilliland and Speck (1977) observed that *Lactobacillus acidophilus* is highly efficient in combating the growth of intestinal pathogens. Inhibited growth of ETEC and other etiologic agents of diarrhoea, including *Campylobacter*, *Salmonella*, *Shigella*, *Staphylococcus* and *Bacillus* have also been reported in some *in-vitro* studies using cereal based products (Svanberg et. al., 1992).

Apart from to the problems faced by child diarrhoea, malnutrition is itself being fairly prevalent among infants. Expensive and inadequate supply of nutritious foods, including commercially manufactured baby foods, has attracted people to take advantage of supplementary foods made from cereals and vegetables

(Akpapunama and Sefa-Dedch, 1995). Many children do not take cereal and legume products with ease due to the bulky nature of porridge (Mensha and Sefa-Dedch, 1991) that can be rectified by traditional fermentation to make them lighter and easy to take (Hesseltine, 1983a; Westby and Gallet, 1991).

Significant health benefits from fermented food products are also gained by adult humans and other mammals. Intestinal hypolactemia or lactose intolerance, due to reduced amount of lactase, causes a series of symptoms due to incomplete digestion of lactose (Jay, 1996). Lactose intolerance causes such symptoms as flatulence, abdominal cramps and diarrhoea (Rosaldo, 1997). Such a problem of lactose malabsorption may be reduced by frequent consumption of fermented products consisting of lactic acid bacteria. Fresh Yogurt fermented by yogurt microorganisms, for instance, has been found to be important milk substitute for lactase deficient patients (Gendrel *et.al.*, 1990).

The value of fermented foods is so immense with respect to milk and milk products. Women, who frequently consume fermented milk products, are reported to have reduced risk of cancer (Biffi. *et al.*, 1997). This occurs due to the activity of lactobacilli in the milk products, which are capable of producing a compound having anti-proliferative effect against the growth of breast cancer cell line. Fermented milk products, also play the role of reducing total cholesterol level by reducing low density lipoprotein (LDL) level (Agerback,

1995). Certain fermented vegetable preparations such as fermented papaya have a prophylactic action against age-related diseases such as cancer, diabetes and other neurological disorders like Alzheimers disease (Imao *et al*; 1998). The fermented products act as anti-oxidants and decrease free radicals related to these complications by reducing the level of lipid peroxide and increasing superoxide dismutase activity

Incidence of coronary diseases, associated with high cholesterol level, can be lowered by consuming fermented yogurt. This contains microorganisms which inhibit the synthesis of cholesterol from acetate (Jay,1996) and the factor, 3-hydroxy-3-methyl glutaric acid, is responsible for this inhibition (Roa *et.al.*,1981).

There are certain items, which are not extensively used directly for food in their natural state due to the presence of some protease inhibitors such as trypsin inhibitors and reduce digestibility of proteins. (Oye yiola, 1991). They also contain flatulence causing oligosaccharides such as raffinose and trehalose (Uzogara,1990). These foods become palatable once they get fermented. In the preparation of tempeh, for instance, the trypsin inhibitor is inactivated and the factors that cause flatulence are eliminated (Hesseltine,1983a ). The beany flavor of dawadawa, a food condiment in Nigeria, is converted by the process

of fermentation into a more palatable nutritious condiment with a sweet nutty flavor (Uzogara, 1990).

### **2.3. Nutritional Value of Fermented foods**

Food fermentations are complex transformations of organic substances resulting from the catalytic action of enzymes native to the substance or produced by the microorganisms in the food (Pederson,1979). Fermented foods, on the other hand, are the result of the activity of a few specialized microorganisms.

Apart from the preservation of available food, there is an increasing need for the production of more food, particularly protein- and vitamin-rich food, by fermentation. Hence fermentation is an important option, in countries where protein supply is scarce and expensive, to upgrade the protein content of various foods (Hesseltine and Wang, 1980). Various low protein vegetable foods can be upgraded for their protein content. A large number of the World's population is said to be vegetarian for economic and cultural reasons. Therefore traditional fermented protein-rich foods provide an excellent opportunity by way of improving the diets of this population (Latunda-Dada, 1997). Among the various attempts to increase the protein levels of vegetables, cassava-based products are very popular particularly in tropical countries. By growing the fungus *Aspergillus niger* for 24 hours on cassava flour with an

initial content of 2%-3% Protein and 80-90% Carbohydrate, it is possible to get a product called 'Gari' having 18%-20% protein and 30%-35% carbohydrate (Latunda-Dada,1997). Most of these changes are brought about by the enzymatic relations of microorganisms, and result in specific transformation of substances (Pedrson, 1979; Hesseltine and Wang, 1980). The Protein content of cassava can also be improved by fermenting it with *Rhizopus* starter culture (Stanton and Walbridge, 1980). Cassava, as a staple food for millions of people in the world, provides an excellent source of calories except that its protein content is extremely low (1-2%). Cassava, or any other substrate rich in starch, therefore, can be upgraded to improve the overall nutritive value by the process of fermentation (Steinkraus, 1983a). The Indonesians, for instance, centuries ago used the mold *Amylomyces rouxii* and a yeast, *Endomycopsis burtoni* to improve the protein level of cassava.

The Importance of fermentation in improving the nutrient quality of other foods such as Tempeh is also very considerable. This widely consumed fermented product in South East Asia is made from soya beans soaked, dehulled, partially, cooked and inoculated with *Rhizopus* mold (Steinkraus, 1983a; 1985). These workers have found out that Indonesian tempeh offers the world a process of making low cost protein-rich meat analog with an excellent nutritional quality due to the process of fermentation. The importance of the molds in tempeh fermentation is the synthesis of enzymes which hydrolyze soya bean

constituents which, apart from improving the texture, flavor and aroma of the product and decreasing anti-nutritional constituents, also improves the nutritional quality of the fermented food (Hachemeister and Fung, 1993).

The nutritional quality of dairy products is also improved by the process of fermentation. Many lactic acid bacteria are involved in such nutritional improvement (Thorenx *et.al.*, 1998). Such fermented milk products as yogurt , Keffir, sour milk provide increased protein utilization and body mass increment to the consumer due to a better protein digestibility of products (Vass *et.al.*, 1984). Fermented dairy products are also good sources of calcium, riboflavin and vitamin B<sub>12</sub> ( Savaiano and Levitt, 1984). The Vitamin content of several other foods can also be improved by fermentation. Except the affluent meat eaters who get their daily vitamin B<sub>12</sub> from meat or milk, most vegetarians face problems in acquiring sufficient vitamin B<sub>12</sub> . It has been found, however, that commercial tempeh made by fermentation invariably contains vitamins B<sub>12</sub> activity due to the specific bacterium that accompanies the fungus during the fermentation. Not only this, even the amount of riboflavin doubles and niacin increases seven times (Steinkraus, 1983b). *Candida* yeasts, *Escherichia coli*, *Aerobacter aerogenes*, *Azotobacter* and *Clostridium acetobutylicum* are widely involved in improving the vitamin content of such foods by fermentation (Steinkraus, 1985)

Other foods whose protein content is highly upgraded by the microbial fermentation include 'Ugba', a fermented oil bean seed in Nigeria (Obeta, 1983), kanji, a fermented porridge, made from whole grain flour of finger millet in semi arid tropics (Antony and Chandra, 1997), miso, a semi solid salty food of the orient made from soya beans, rice or barley and salt (Yokotsuka, 1985) and many others for which the wide application of fermentation in improving the nutritional quality is greatly acknowledged.

The availability of certain minerals can also be enhanced by microbial fermentation of some foods (Latunda-Dada, 1991), Fermentation of cereals, for instance, improves the availability of iron in tropical countries. Fermented Soya bean has been reported to have increased availability of Zinc by the digestion of phytic acid (Hirabayashi *et.al.*, 1998)

#### **2.4. Condiments**

No matter how nutritionists and health enthusiasts may wish, it is the flavor and appearance of food rather than the vitamin content that wins the complement at the dinner table (Coultate, 1995). Most foods hence acquire such pleasant characteristics due to the presence, along with them, of a condiment, something which adds flavor to other food's and makes them more tasty and enjoyable (Hesseltine, 1983a). Many condiments can serve as food supplements due to their high nutrient content. 'Iru', for example, is an important condiment in

Nigeria which also serves as low cost meal substitute. Yokotsuka (1985) reported that as the rising population causes more demand for animal protein, iru or a similar vegetable protein used as condiment is important to supplement the protein intake.

The value of condiments can be traced back in time when people in the Orient have enriched the flavor of cheese by fermenting it with some *Pencillium* molds (Yokotsuka, 1985). People in the Orient have developed mechanisms to add to the flavor of fish, proteintious beans, meat and some cereals by fermenting them with proteolytic and amylolytic enzymes produced by *Aspergillus* or other molds.

Many condiments are assuming greater importance in several developing countries not only as flavoring agents but also as protein supplements, since the majority of the population can no longer afford the high cost of edible protein (Odunfa and Oyewole,1986; Ogbadu *et.al.*, 1990). Certain condiments made from fermented legume seeds have higher protein and tannin content in addition to other nutritive qualities such as increased level of zink, sodium and phosphorus (Obizola and Atu, 1993).

In most parts of Africa protein rich foods are often fermented to make condiments. The use of hydrolyzed vegetable proteins has long been used as flavoring agent. (Odunfa, 1985b). In Ethiopia, for instance, a popular food

condiment known as 'Siljo' is made from fermented legumes, Safflower, (*Carthmus tinctorius*) and horse bean (*Vicia faba*) flours which is consumed as a side dish to any one of the major legume based sauces along with injera, a pancake and its microbiological properties have been worked out (Tetemke Mehari and Mogessie Ashenafi, 1995).

Quiet often than not it is the wish and pleasure of every one all over the world to have meat in the diet despite the inadequate supply of meat. The Chinese, thousands of years ago, showed the world how to produce meat-like flavors from vegetable proteins, one of the great discoveries in food science (Steinkraus, 1983a). Of the many types of such discoveries the production of Soy sauce, consumed widely in China, Japan and other Asiatic countries takes the upper hand in the history of condiment fermentation (Luh, 1995). This all-purpose seasoning agent characterized by a pleasant aroma and complex flavor is made by two step process of fermentation from wheat flour and soya beans with a mixture of molds, yeasts and bacteria.

In a similar fashion the Indonesians developed another condiment, tauco, a meat flavored soya bean paste or liquid used with various other foods including fish or in vegetable soup (Winaro, 1979). Thus the importance of condiments in improving the flavor and nutritional quality of the diets of many people of the world can not be under estimated.

### **2.5. Traditional Fermentations**

Traditional fermentations are those mediated by the hydrolytic influence of indigenous flora or deliberately added enzymes derived from the microbial activities on the substrates. The process employs the entire natural microflora that could function under the varied environmental and non-sterile conditions (Latunda-Dada, 1997) and uncontrolled inoculation (Sani,1993). These traditional fermentations have been used for centuries (Hesseltine and Wang, 1980).

The majority of African diets are based on traditional fermented foods. While most of these traditional foods serve as main course meals, others are beverages or highly prized condiments (Odunfa, 1985a). The fermentation of carbohydrate-rich materials usually is employed to produce main meals and beverages while condiments are more often produced by the fermentation of vegetable proteins.

The majority of traditionally fermented African foods such as alcoholic starchy foods, beverages, fermented vegetable proteins and animal proteins are fermented by a few groups of yeasts and bacteria (Ógbadu and Okagbue, 1988), while fermented foods of South-East Asia, in general, are mainly fermented by molds.

Fermented foods in mid-Asia, the Middle East and Africa are acid products prepared by bacterial and yeast fermentations of cereals such as millet, sorghum, maize and wheat. In the Middle East, this is produced from milk while in India and Pakistan it is formed from locally grown legumes (Hesseltine and Wang , 1980).

Traditional fermentations in Africa which make use of mixed culture of spontaneous fermentation have resulted in the selection, over the centuries, of certain microorganisms that grow together to produce a desirable product (Sanni, 1993). This is not, however, to deny that such mixed cultures and uncontrolled fermentations have brought about recurring variations in the quality of the fermented products (Latunda-Dada, 1991).

## ***2.6. Fermented Products of Ethiopia***

Fermented foods are essential parts of the human diet in many parts of the world and Ethiopia is no exception. Among the many food items in Ethiopia the most common and the major staple foods are kotcho and injerra (Berhanu Abegaz, 1985; Mogessie Ashenafi, 1994). The fermentation is spontaneous and usually initiated with members of enterobacteriaceae which are then succeeded by lactic acid bacteria (Berhanu Abegaz, 1985). Studies show that food borne pathogens in this products are inhibited by lactic acid bacteria

during the fermentation of these products (Ayele Nigatu and Berhanu Abegaz, 1994).

In almost the majority of rural Ethiopia and most urban areas fermented milk products are frequently consumed. Ayib, a traditional Ethiopian cottage cheese, has a low pH and does not allow the growth of food-borne pathogens, although the high load of acid tolerant microorganisms makes its keeping quality very poor (Mogessie Ashenafi, 1990). A similar dairy product, Ergo, a sour milk, is consumed in its fermented state (Mogessie Ashenafi, 1993). Ethiopia is also known for its various types of traditional fermented beverages produced mostly on a fairly small scale and usually for local consumption. These include *borde* and *shamita* (Mogessie Ashenafi and Tetemke Mehari, 1995; Ketema *et al.*, 1998, 1999), *tella* (Samuel Sahle and Berhanu Abegaz, 1991) and many others. Among the various condiments, a series of studies have been made on *siljo*, a fermented legume condiment, with respect to fermentation and safety (Gulilat *et al.*, 1997; Tetemke Mehari and Mogessie Ashenafi, 1995).

## **2.7. Fermentation and The Food Industry**

It has been mentioned that fermented foods constitute the major staples, adjunct staples, beverages and condiments in most African diets and the diets of other developing and developed nations. Nowadays, there is a growing concern on indigenous foods to improve the nutritional value by employing better methods of production (Hesseltine, 1983a).

To arrive at such a goal is difficult if the effort depends on traditional fermentations at household level. Most techniques employed in traditional fermentations, even at the cottage level, depend upon simple materials, chance or natural inoculum, unregulated conditions, sensory fluctuations in taste and flavor and poor durability of the finished products (Latunda-Dada, 1997).

It is here that fermented food industries have greater importance. The huge international enzyme and food industry today can be traced directly back to the Japanese *shoyu* (soy sauce) fermentation and are going at a faster rate (Steinkrause, 1983 a) The mono-sodium-glutamate industry (MSG) and the nucleotide flavor-enhancing industry are also outgrowths of the soy sauce industry. Today the fermented food industry is growing at a faster rate.

The food fermentation industry, as opposed to traditional fermentation processes, has such requirements as good quality raw materials efficiently graded and stored at optimum conditions, simple equipment and attractive packaging. Large scale productions of fermented foods involving the use of heavy machinery, on the other hand, is not only expensive and demands high capital input but is also too expensive for most people in developing countries (Latund Dada, 1997).

In a situation where large scale industrially fermented foods are expensive, small scale agro-allied cottage industries should take the upper hand. Many countries possess a wide range of fermentable indigenous staple food that are used as raw material for such cottage industries. Today Biotechnology is becoming a latest tool employed to this effect to solve problems of production and to upgrade and modernize traditional food fermentation (Luh, 1995).

### 3. MATERIALS AND METHODS

#### 3.1. Sample Collection and Processing

All the ingredients items required for laboratory fermentation of 'awaze' and 'datta' were purchased in bulk from the market in Addis Ababa. The ingredient bought for 'awaze' preparation were red sweet pepper, "Dube berbere" (*Capsicum annum*), Garlic, "Nech shinkurt" (*Allium ursinum*), Ginger, "Zingible" (*Zingibel officinale*), Sweet basil, "Besobila" (*Ocimum sanctum*), Rue, "Tenadam" (*Ruta Chalepensis*), Cinnamon (*Cinamomum zylanicum*), Clove (*Eugenia caryophylla*), Ethiopian caraway "Nech azmud" (*Trachyspermum copticum*), Ethiopian cardamon, "Korerima" (*Aframomum angustifolium*), dry sweet basil (*Ocimum sanctum*) and salt. For 'datta' preparation, the main ingredient was small green pepper (*Capsicum frutescense*), garlic, fresh sweet basil and rue (*Ruta chalepensis*). Once all these ingredients were ready, the pre-processing of 'awaze' ingredients was performed by knowledgeable housewives following the traditional methods as described in Fig.1 and Fig. 2.

All the seeds of the sweet pepper were first discarded and the fruits were washed with cold water and sun dried . The dried seedless fruits of the sweet pepper were gently pulverized with a wooden mortar and pestle. Fresh undried ginger and garlic were peeled, washed and mixed together with small

proportions of fresh sweet basil and the seeds of rue. These were then mixed with the already dried and pulverized sweet pepper and kneaded together. The whole kneaded mixture was left in a container for an overnight to form a solid mash. The next day the mash was sun-dried after being spread on a clean surface. This was set aside until the next processing.

Small proportions of the dry spices including clove tree, Cinnamon tree, Ethiopian caraway, Ethiopian cardamon and sweet basil together with certain amount of salt were gently heat-treated separately on a metal pan. The heat-treated spice mixture was mixed with the kneaded and dried pepper spice mixture and was dry milled. The milled mixture was sieved through a fine wire mesh screen and the pomace left on the sieve was discarded.

To study the laboratory fermentation of '*awaze*', a certain amount from the milled pepper-spice ingredient was mixed with warm water in a 500 ml capacity screw cap bottle, stirred well until it acquired a thick jelly like consistency and left to ferment at ambient temperatures.

*'datta'* was prepared following traditional methods. The small green pepper together with its seed was washed and cut into pieces. Garlic and ginger, in small proportion, were peeled, washed and cut into pieces.

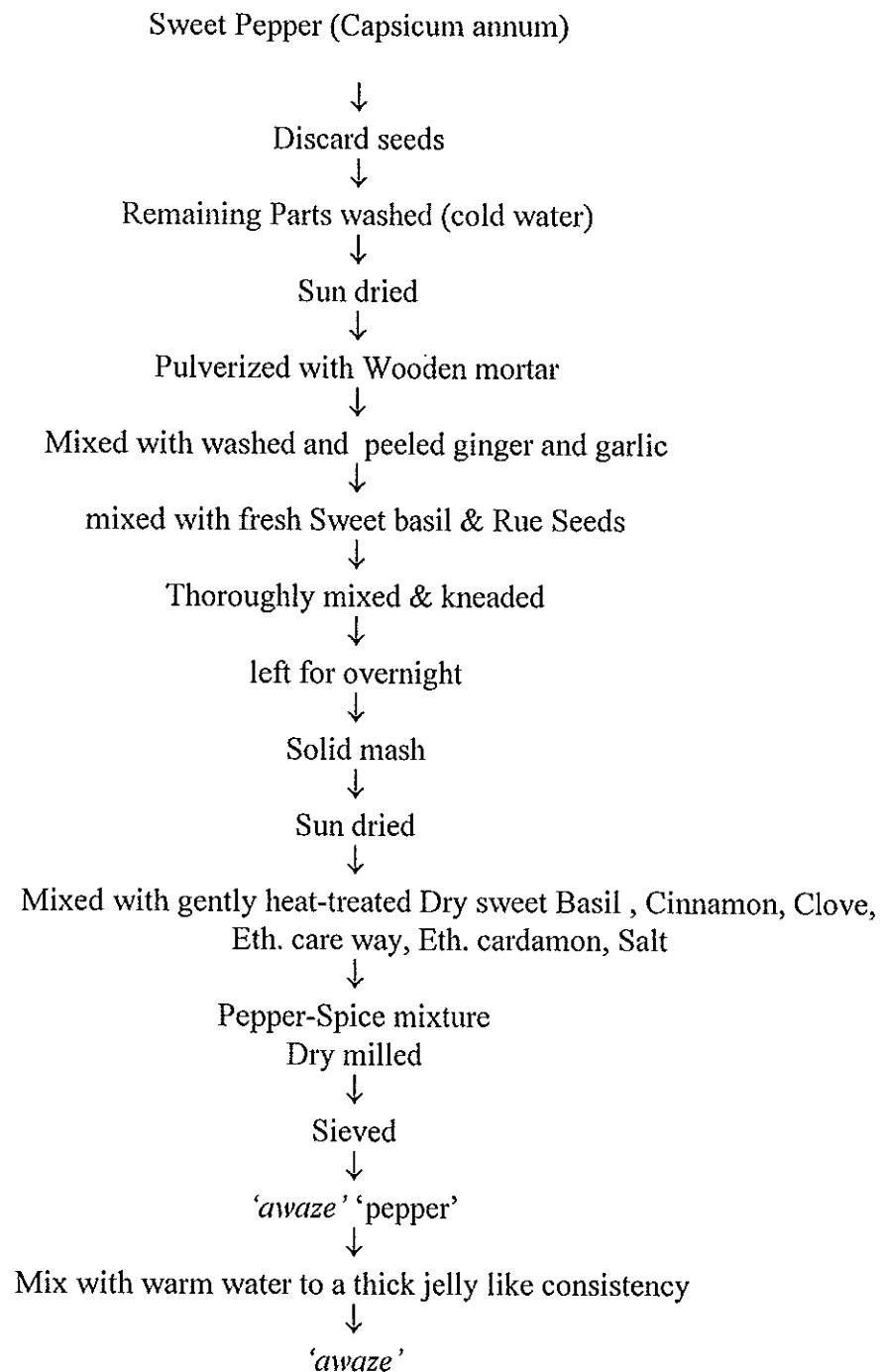


Fig. 1. Flow chart of 'awaze' processing

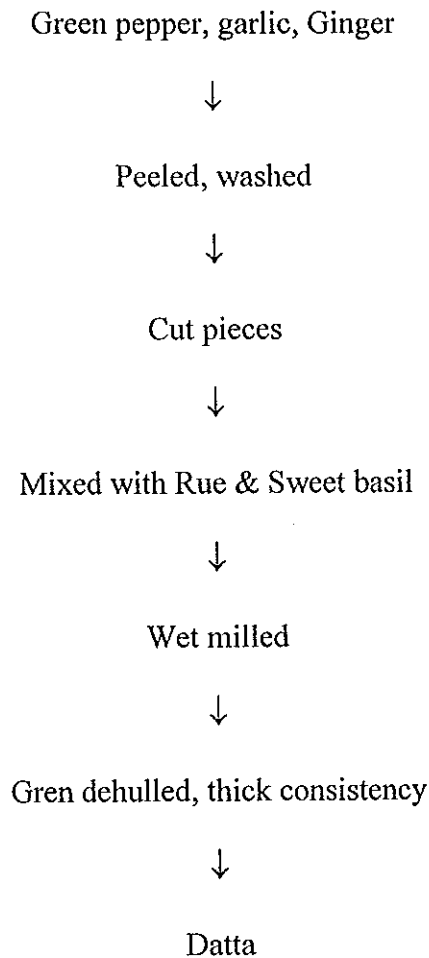


Fig. 2. Flow chart of Datta preparaton

The pepper, garlic and ginger were mixed with trace amounts of fresh sweet basil and seeds of rue. The mixed ingredients were wet-milled on a flat smooth traditional stone mill that operated manually. The milling was performed with gradual addition of the mixture and coarse salt to finally obtain a greenish, dehulled product with thicker consistency than '*awaze*'. It was then transferred into a 500ml screw capped bottle to ferment.

### **3.2. Enumeration of Microbial Groups**

One gram of the mixed raw ingredients and the fermenting 'awaze' and 'datta' were separately taken and mixed with 9 ml 0.1% peptone water. This was vigorously mixed with a vortex and serial dilutions were made for microbial counts. A volume of 0.1 ml of appropriate dilutions were spread-plated on different culture media as described below. Both 'awaze' and 'datta' were allowed to ferment in 500 ml capacity screw cap bottles under laboratory conditions. Samples (1 g) were aseptically withdrawn for further processing.

#### *Aerobic Mesophilic counts (AMC)*

From appropriate dilutions, 0.1 ml aliquots were spread plated in duplicates on pre-solidified surfaces of nutrient agar (NA) (Oxoid) plates.

#### *Aerobic bacterial spore count*

Homogenized samples were kept at 80°C for 10 minutes in a water bath to kill vegetative cells. Then 0.1 ml of appropriate dilutions was spread-plated in duplicates on pre-dried surfaces of nutrient agar (Oxoid) plates. The seeded culture plates were incubated at 30-32°C for 24-48 hours for colony counting.

#### *Counts of staphylococci*

From appropriate dilutions 0.1 ml aliquots were spread plated in duplicates on pre-dried surfaces of mannitol salt agar (Oxoid) plates. The culture media were

incubated at 30-32° C for 36 hours after which yellow colonies were counted as *Staphylococci*.

#### *Coliform counts*

From appropriate dilutions 0.1 ml aliquots were spread plated in duplicates on pre-solidified surfaces of violet Red Bile agar (Oxoid) plates. The seeded culture plates were incubated at 30-32° C for 20-24 hours after which purplish red colonies surrounded by reddish zone of precipitated bile were enumerated as coliforms.

#### *Counts of Enterobacteriaceae*

From appropriate dilutions 0.1 ml aliquots were spread plated in duplicates on pre-solidified surfaces of Violet Red Bile Glucose agar (Oxoid) plates. The seeded culture plates were incubated at 30-32° C for 20-24 hours after which pink to red-purple colonies with or without haloes of precipitation were counted as members of family Enterobacteriaceae.

#### *Lactic acid bacteria counts*

A volume of 0.1 ml of appropriate dilution was surface plated in duplicates on pre-dried surface of de Mann Rogosa and Sharp (MRS) agar (Merck) and plates were anaerobically incubated at 30° C for 24 hours for counting.

#### *Mould and yeast counts*

From appropriate dilutions, 0.1 ml aliquots were spread plated in duplicates on pre-solidified Chloramphenicol Bromophenol Blue agar made from the following ingredients: yeast extract, 5 g; dextrose, 20 g; chloramphenicol, 0.1 g; bromophenol blue, 0.01 g; agar, 15 g; distilled water, 1000 ml). The seeded culture plates were incubated at 25-28° C for three to five days. During enumeration yeasts were distinguished from moulds by using stereoscopic microscope. Thus, smooth (non-hairy) colonies and without extension at periphery (margin) were quantified as yeasts, whilst big, spreading and hairy colonies at margin and surface were counted as moulds.

To check for the sterility, one plate was incubated at 37° C overnight from each batch of the solid medium used before undertaking inoculation.

### ***3.3. Flora analysis***

After enumeration of aerobic mesophilic bacteria, about ten to twenty colonies were picked randomly from countable plates and inoculated into tubes containing about 5 ml nutrient broth No 2 (Oxoid). These were incubated at 37° C overnight. Cultures were purified by repeated plating and were characterized to the genus level and various bacterial groups using the following tests:

#### *Cell morphology*

From overnight pure broth culture a hanging drop technique was employed. The preparation was observed under light microscope using oil immersion objective. The morphological criteria considered during the observation were:

Cell shape: - Regular: rods, cocci, coccoid forms  
- irregular: branched, coryneforms, pleomorph

Cell arrangement:- Singles, pairs, clusters, chains, tetrads

Motility:- Motile, non-motile

Spore staining was employed to check whether the isolate under question was endowed with endospore or not.

#### *KOH Test*

This test was performed as proposed by Gregersen (1978). One or two drops of 3% KOH solution were placed on a clean microscope slide. A colony was picked with a sterile bacteriological wire loop and stirred in the KOH solution for 10 seconds to 2 minutes and the inoculating loop was then raised slowly from the mass. When KOH solution became viscous, the thread of slime followed the loop for 0.5 to 2 cm or more. Typically, this was observed in Gram-negative bacteria. In case of no slime and the watery suspension did not follow the loop, the reaction was negative and this was seen in Gram-positive bacteria

#### *Oxidation-Fermentation (O/F) test*

The utilization of glucose by each isolate was assessed by O/F test as suggested by Hugh and Leifson (1953) to identify microorganisms that metabolize glucose fermentatively or oxidatively or that do not utilize glucose by either way.

Ingredients (g/l): Peptone, 2g; yeast extract, 1g; NaCl, 5g;  $K_2HPO_4$ , 0.2g; glucose, 10g; Bromothymol blue, 0.08g; agar, 2.5g; distilled water, 1000 ml, pH, 7.10.

The freshly prepared medium (15ml amounts in 18 x 180 mm test tubes) was immediately cooled under tap water and inoculated by stabbing with a sterile straight wire to the bottom. Acid formation and growth regions were interpreted after 2 and 5 days of incubation at 37°C.

#### *Catalase test*

Young colonies were flooded with a 3% solution of  $H_2O_2$ . The formation of bubbles indicated the presence of catalase.

#### *Cytochrome oxidase test*

This test was conducted following the method outlined by Kovacs (1956). Freshly prepared reagent A and B were mixed in the ratio of 2:3 immediately before use.

Reagents:

A) 1%  $\alpha$ -naphthol in absolute ethanol

B) 1% N,N-dimethyl-p-phenylene diammonium chloride in distilled water

After flooding the young (24 hours) colonies with the mixture Nutrient Agar plates, appearance of a blue color on the colonies within 30 seconds to 2 minutes indicated a positive reaction. Any very weak or dubious reaction that occurred after 2 minutes was ignored.

Lactic acid bacteria isolated from MRS were further characterized into homo and hetero-fermentative groups . Ten colonies were randomly picked from MRS plates and transferred into MRS broth (Merck) containing 2% glucose with 0.001% bromocresol purple indicator as used by Adegoke *et.al.*, (1988). To help detect the production of gas during glucose metabolism, inverted Durham tubes were put in the MRS broth.

All isolates were identified according to Collins and Lyne (1976) and Mitruka, 1977.

### **3.4. Determination of pH and Titratable acidity**

pH measurement of the fermenting 'awaze' and 'datta' was determined with pH meter (Beckman). Five gram of fermenting sample was dissolved in 20 ml distilled water and pH was read by immersing the electrodes in the sample (Ogbadu and Okagbuye, 1988). For the determination of titratable acidity, 5g of the fermenting sample was dissolved in 20 ml distilled water through whatman No. 1 filter paper. To 20 ml of the filtrate 2.3 drops of phenolphthalein was added as indicator. This was titrated against 0.05 M NaOH to the end point of phenolphthalein (Antony and Chandra, 1997). Titratable acidity was expressed as g lactic acid/100g of 'awaze' or 'datta' and was calculated using the following formula.

$$TA = \frac{M_{NaOH} \times ml \ NaOH \times 0.09 \times 100}{ml \ sample}$$

### **3.5. Determination of inhibition of *Salmonella typhimurium* in fermenting 'awaze' and 'datta'**

The microbial safety of the fermentation of the two condiments was assessed by determining the growth potential of *Salmonella typhimurium* in the fermenting samples. A volume of 400 ml of 'awaze' and 'datta' were inoculated with an overnight culture ( $10^8$ - $10^9$  cfu/g) of *Salmonella* test strain. After the inoculated samples were thoroughly mixed for 10-15 minutes, 1g of sample was taken and serial dilutions were made every eight hours. Appropriate

dilutions (0.1 ml) were spread-plated on nutrient agar and incubated at 37<sup>0</sup> for one hour to enhance the metabolic recovery of injured cells . After the one hour incubation the plates were further over-laid with SS (*Salmonella-Shigella*) agar and were incubated at 32<sup>0</sup> C for 24-36 hours for colony counting.

### **3.6. Biochemical Changes During 'awaze' and 'datta' Fermentation**

#### **3.6.1. Determination of changes in the level of reducing sugar**

Reducing sugar changes throughout the course of the fermentation were detected by the DNS-colorimetric methods as described by Miller (1959) and James (1995) with slight modification.

#### *Preparation of extracts*

Three grams of fermenting sample was aseptically withdrawn and added into a conical flask and 50 ml distilled water was added to it. This was gently warmed with continuous stirring for 10 minutes and was filtered with glass wool into a 100ml volumetric flask. The residue was washed into the volumetric flask with small amounts of water and mixed well by inversion. Ten ml of this solution was diluted to 250ml distilled water in another volumetric flask and again mixed well by inversion. This was used for analysis.

### *Standard glucose preparation and absorbance measurement*

For the preparation of standard glucose solution 15 mg/ml stock solution of glucose was diluted to get solutions of 0.25, 0.5, 1.0, 1.25 and 1.5 mg. glucose /ml. One ml of these standard glucose solutions was pipetted into 5 labelled test tubes. A volume of 1.0 ml distilled water was pipetted into another test tube and served as a blank. Then 1 ml Dinitro-salicylic acid (DNS) reagent and 2.0 ml distilled water was added to each one of the tubes. DNS reagent was prepared by dissolving 5g of dinitro-salicylic acid , 1g phenol, 0.3g sodium sulphite, 100g potassium sodium tartarate and 5g NaOH in 500 ml distilled water. The reagent was kept in brown bottle until used.

### *Preparation of Calibration graph*

For Calibration graph preparation all tubes were heated in boiling water bath for 5 minutes to allow the reaction between glucose and DNS to occur. After cooling, the volume of each tube was adjusted to 20 ml to get a final concentration of 0.25, 0.5, 1.0, 1.25 and 1.5 mg glucose/20 ml. Calibration graph was made by plotting absorbance against mg glucose per 20 ml at 540 nm.

To determine the total reducing sugar, 1ml from the extract prepared above was added into a test tube. To this were added 2.0 ml distilled water and 1ml DNS reagent. The tube was heated in boiling water bath for 5 minutes. After cooling and adjusting the volume to 20 ml, absorbance was read at 540 nm using spectro-photometer (spectronic 2001). The amount of reducing sugar in

the sample was extrapolated from a standard curve prepared using known concentration of glucose as a standard.

### **3.6.2. Determination of Protein in Fermenting 'awaze' and 'datta'**

The change in the protein content of the fermenting samples was determined using formol titration (James, 1995) with some modification.

Sample (5g) was filtered in 20ml distilled water through Whatman No.1 filter paper. Ten ml of this filtered extract was mixed with 1 ml phenolphthalin and 0.4 ml saturated potassium oxalate solution in a small conical flask. After about 2 min, this was titrated to a pink color against 0.1M NaOH solution. Then 2 ml of formaldehyde (37%) was added and the titration was continued for the second time to the end -point of phenolphthalein. The amount of NaOH required for the second titration only was recorded for calculation.

The amount of protein was determined using the following relationship.

$$\% \text{ Protein} = \text{Aldehyde value} \times 0.17$$

where aldehyde value is the volume of 0.1M NaOH required per 100 ml of sample for the reduction of the acidity produced by the formaldehyde.

## 4. RESULTS

### **4.1 Microbial flora of Ingredient mixture of 'awaze'**

The total aerobic mesophilic count in 'awaze' ingredients was  $1.1 \times 10^6$ , and this was completely dominated only by *Bacillus* spp. The aerobic spore count was  $7.0 \times 10^5$  cfu/g and counts of lactic acid bacteria was  $4.5 \times 10^4$  cfu/g. Yeasts and other groups of microorganisms were below detectable levels in the initial mixture.

### **4.2. Microorganisms in Fermenting 'awaze' and 'datta'**

A marked increase in count of aerobic mesophilic bacteria was noted as early as 8 hours of fermentation in fermenting 'awaze'. Maximum count was reached at 24 hours and the count declined thereafter (Fig. 2). No increase in count of aerobic spore formers was noted, and rather a slight decrease was noted as the fermentation proceeded further. The lactic acid bacteria increased steadily until they reached their maximum count of  $2.5 \times 10^{11}$  cfu/g at 104 hours (Fig 3).

Yeasts reached countable levels only at 168 hours (7<sup>th</sup> day) and kept on increasing until they reached levels as high as  $2.5 \times 10^6$  cfu/g at 296 hours (14<sup>th</sup> day), when the fermentation was about to reach its final stage.

The process of 'datta' fermentation required much less time than was required by 'awaze' fermentation. The microbial dynamics was also a bit different.

Table 1. Microorganisms isolated during the fermentation of Awaze and Datta

Condiment	Functional Group	Taxonomic Group
Datta	AMC	Bacillus spp., EB, Colliforms, Staphylococci
	ASF	Bacillus spores
	LAB	—
Awaze	AMC	Bacillus spp.
	ASF	Bacillus spores
	LAB	—
	Yeasts	—

AMC= Aerobic Mesophilic Count, ASF= Aerobic Spore Formers, LAB= Lactic Acid Bacteria, EB= Enterobacteriaceae, —= Taxonomic group not known

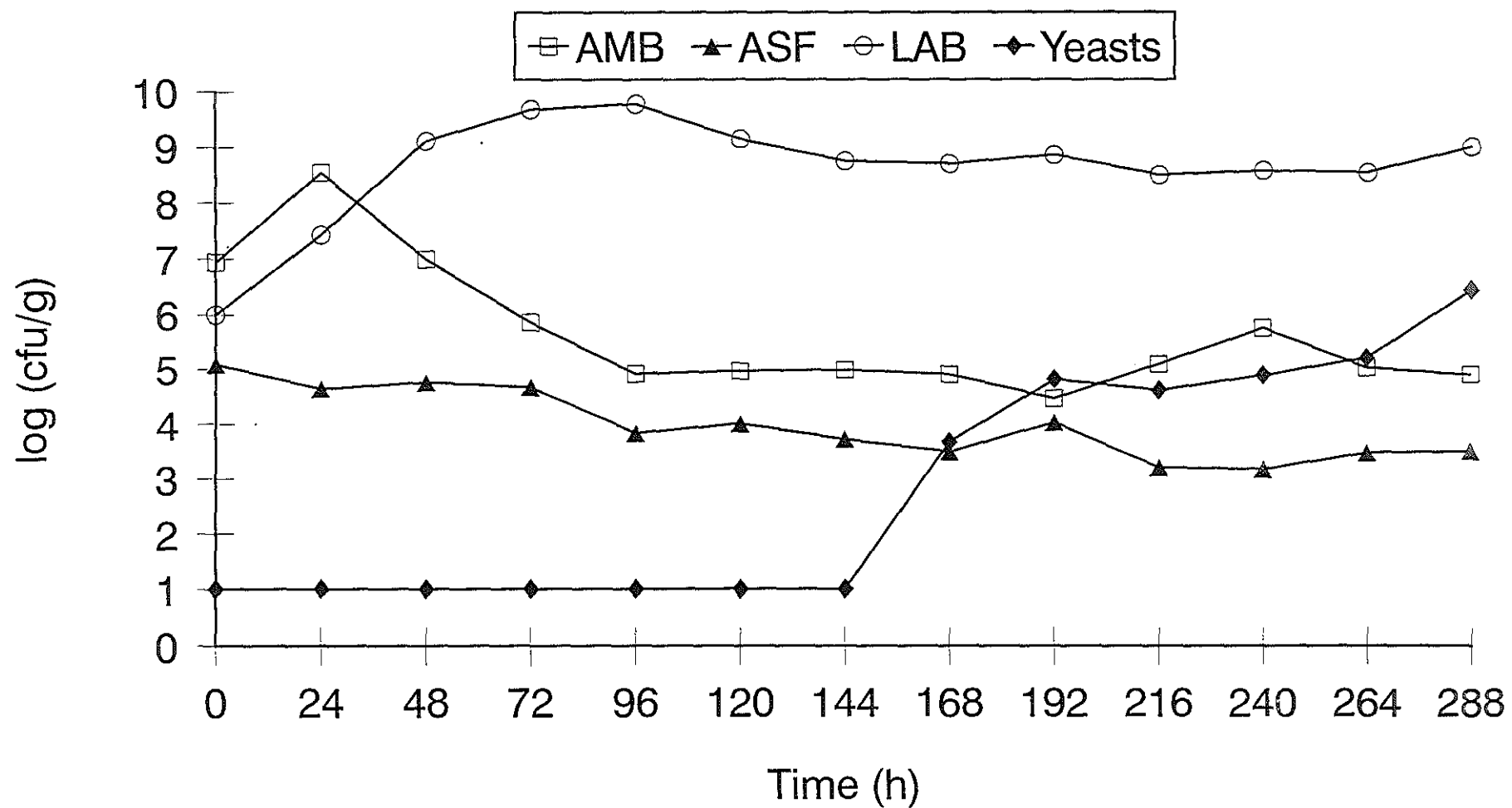


Fig 3. Growth pattern of aerobic mesophilic bacteria (AMB), aerobic spore forms (ASF), lactic acid bacteria (LAB) and yeasts during awaze fermentation

The aerobic mesophilic count and count of aerobic spore formers remained more or less unchanged as there was no marked difference in count between the first and the seventh days. The initial count of coliforms and members of enterobacteriaceae also decreased at a markedly high rate and they were below detectable levels after 32 hours of fermentation (Fig. 4). Staphylococci survived the seven-day fermentation although no increase was noted in their count. Yeasts were not detected throughout the fermentation. The only bacterial groups which increased in number steadily were the lactic acid bacteria. They initiated the fermentation at a level of  $7.1 \times 10^4$  cfu/g and reached the maximum count of  $1.2 \times 10^9$  cfu/g at about 152 hours (Day 7) (Fig. 4).

During the fermentation of 'awaze', the aerobic mesophilic bacteria were dominated mainly by *Bacillus* spp (97.5%) followed by a small proportion staphylococci (data not given). In the case of 'datta' fermentation, too, *Bacillus* spp. were the most dominant, although staphylococci, micrococci and Enterobacteriaceae constituted the dominant microflora (Table 2).

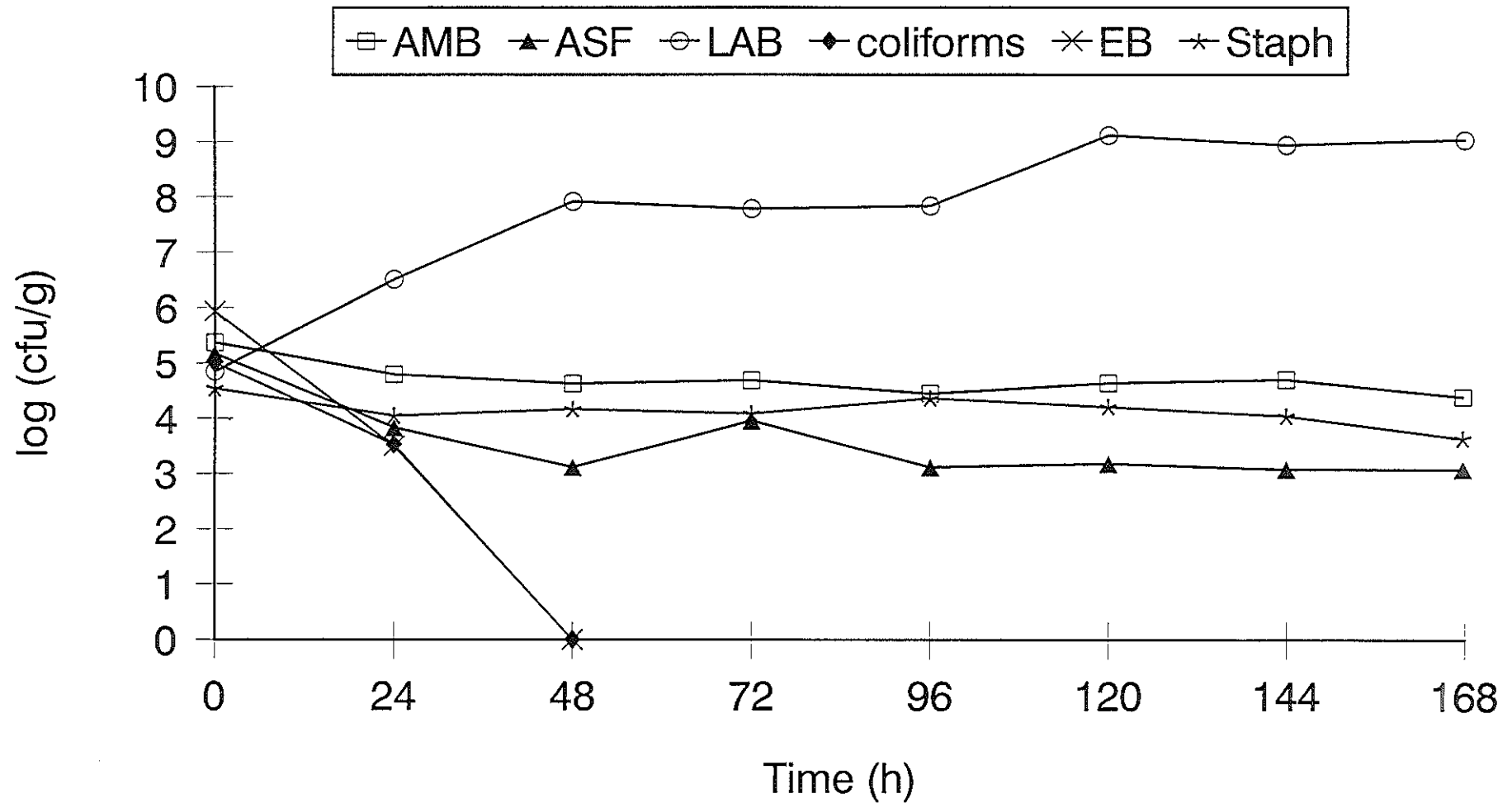


Fig 4. Growth pattern of aerobic mesophilic bacteria (AMB), aerobic spore forms (ASF), lactic acid bacteria (LAB), coliforms, Enterobacteriaceae (EB) and staphylococci (Staph) during datta fermentation

Table 2. Distribution of dominant microorganisms in the fermentation of 'datta'

Fermentation Time (h)	Mean AMC*	Number of isolates	Number of isolates			
			I	II	III	IV
0	5.27	11	5	2	1	3
32	4.83	10	4	2	2	2
80	4.52	10	10	-	-	-
128	4.59	10	7	1	2	-

\*, Counts are in log c.f.u/g

I, *Bacillus* spp. II, *Staphylococcus* spp. III, *Micrococcus* spp. IV, Enterobacteriaceae

AMC = Aerobic mesophilic count

#### 4.3 Dynamics of lactic acid bacteria during 'awaze' and 'datta' fermentation

Both homo-fermentation and hetero-fermentative groups of lactic acid bacteria were represented in the fermentation of the two condiments (Table 3). 'Awaze' fermentation is initiated and carried out dominantly by heterofermentative lactic acid bacteria whose number increased from 6.00 log c.f.u/gm at 0h to 8.92 log c.f.u. at 56hrs. At 96 hours the homo-fermentative lactic acid bacteria dominated the fermenting population and they reached counts as high as  $7.1 \times 10^9$  cfu/g. This level was maintained until 144 hours and then decreased by 1 log unit until the termination of the fermentation. On the other hand, the lactic acid bacteria which initiated 'datta' fermentation were the homolactics. They dominated the fermenting flora until 32 hours without actually reaching a markedly high count ( $4.1 \times 10^6$  cfu/g). Thereafter, the heterolactics took over and grew to the level of  $10^9$  cfu/g at termination of the fermentation.

Table 3. changes in counts log (c.f.u./gm) of Lactic acid bacteria during 'awaze' and 'datta' Fermentation

Time (h)	'Awaze'			'Datta'		
	pH	homo Fermentative	hetero-fermentative	pH	homo fermentative	hetero fermentative
0	5.6	<4	6.0	5.56	4.85	<3
24	5.3	<5	7.43	5.18	6.5	<5
48	4.5	<7	9.11	4.93	<6	7.91
72	4.43	9.14	9.5	4.71	<6	7.78
96	4.36	9.0	<5	4.69	<6	7.83
120	4.31	9.14	<5	4.63	<6	9.11
144	4.09	8.74	<5	4.61	<6	8.93
168	3.94	8.69	<5	4.61	<6	9.03
192	3.92	8.85	<5	END	END	END
216	3.86	8.49	<5			
240	3.86	8.56	<5			
264	3.82	8.53	<5			
288	3.78	8.99	<5			
296	END	END	END			

#### **4.4. pH and Titratable Acidity**

'Awaze' and 'datta' fermentations had similar patterns of pH and titratable acidity changes at the beginning but differed markedly as the fermentation continued. A steady decline in pH was observed in both fermentations and initial pH values were similar in both. In the case of 'awaze' fermentation, the major drop in pH was noted between 24 and 48 hours, where it decreased by 0.8 units (Fig 5). This was accompanied by a major leap in titratable acidity which increased from 0.13% at 24 hours to 0.18% at 48 hours. Lowest pH achieved during 'awaze' fermentation was 3.67 while the maximum titratable acidity was 0.38% at 14thay. In 'datta' fermentation, decrease in pH and increase in titratable acidity more or less steady until 96 hours and appeared to stabilize thereafter at pH values of 4.6 and titratable acidity of 0.14%.

#### **4.5. Microbial safety of the Fermenting Products by determination**

##### **of inhibition of *Salmonella typhimurium***

The inhibition of the challenge organism inoculated in the two fermenting samples is presented in Table 4. A gradual decrease was observed in the count of the challenge strain in fermenting 'awaze' before it reached counts below detectable levels before the completion of the fermentation. *S. typhimurium* decreased by over 2 log units in the first 24 hours of fermentation and this continued gradually until it reached undetectable levels at 48 h. The inhibition

was much faster in the case of '*datta*' fermentation, where it was rapid and inhibition was noted as early as 24 h.

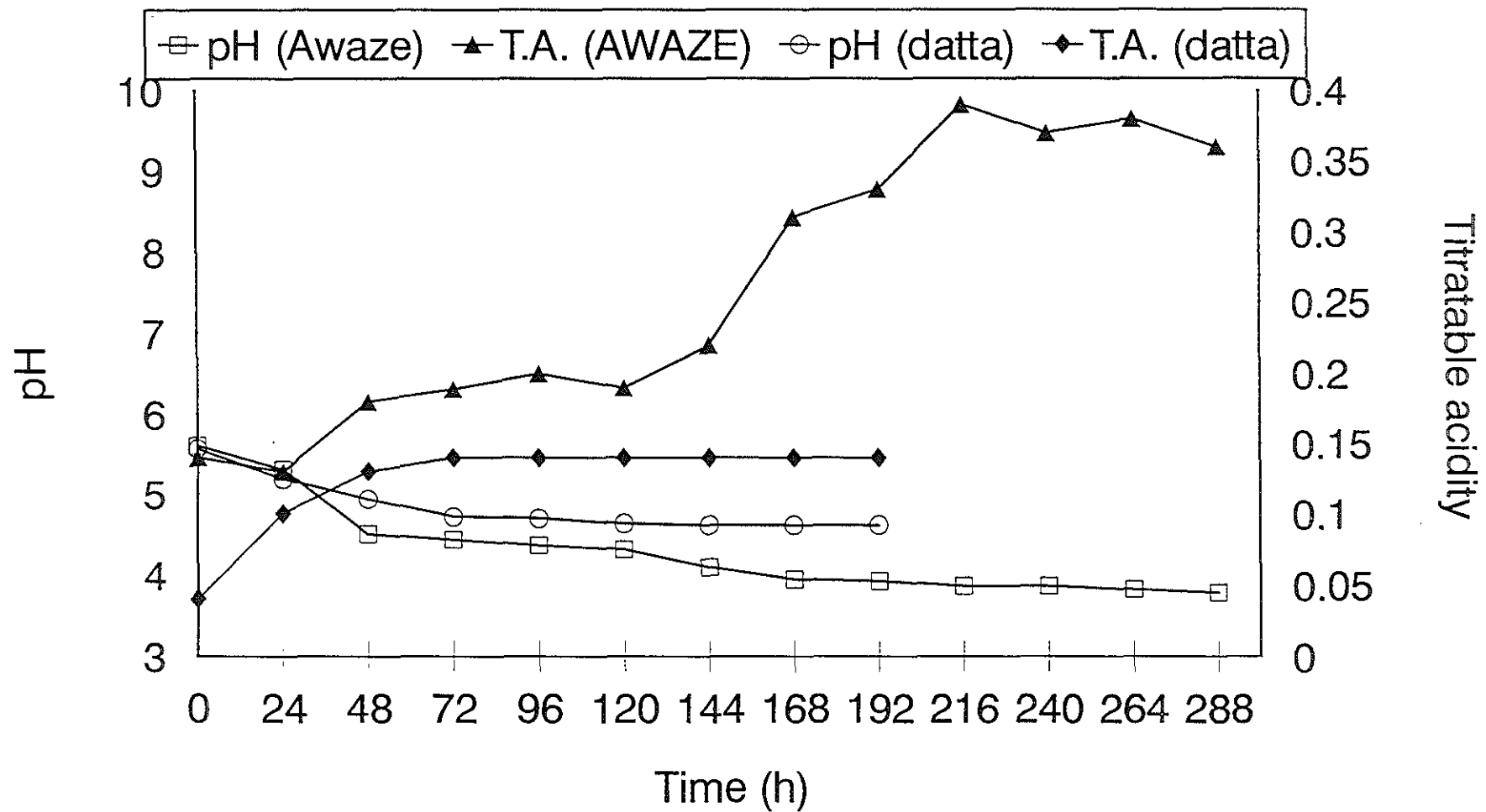


Fig 5. Changes in pH and titratable acidity during awaze and datta fermentation

Table 4. Counts (Log c.f.u/gm) of *Salmonella typhimurium* test strain in the Fermentation of 'awaze' and 'datta'

Time (h)	'awaze'	'datta'
0	6.5	5.69
8	5.50	3.64
24	4.25	2.60
32	3.39	<1
48	2.9	<1
56	<1	<1
64	<1	<1

#### 4.6. Biochemical Changes During 'awaze' and 'datta' Fermentation

In 'awaze' fermentation, the reducing sugar increased in the first 24 hours and declined afterwards sharply up to 48 hrs. It again pick up at 72 hrs. with a progressive decline up to 152 hrs. It then started to rise again around the completion of the fermentation. For 'datta' the changes in reducing sugar level were not so marked except that there was a little increase at the 8hrs. and declined upto 72 hrs. after which it stabilized with no change (Table 5)

The Protein content of fermenting 'awaze' was 0.17% at 0h and decreased to 0.16% up to 104 hrs. It again increased to 0.17% to finally stabilize at 0.16%.

In 'datta' there was more protein content although the Changes during the fermentation are not significant. Upto 56 hrs. of fermentation the level of protein was 0.25% and from 72 hrs. onwards it declined to 0.23% with no further change (Table 6)

Table 5- changes in Concentration of reducing sugar during 'awaze' and 'datta'

Fermentation

Time	'awaze'		'datta'	
	*Absorbance 540 nm	*Concentration mg/20ml	Absorbance 540 nm	*Concentrati on mg/20ml.
0	0.109	1.18	0.012	0.31
8	0.117	1.26	0.007	0.36
24	0.119	1.25	0.007	0.27
32	0.013	0.31	0.007	0.23
48	0.004	0.24	0.002	0.22
56	0.019	0.37	0.003	0.23
72	0.11	1.19	0.001	0.21
80	0.076	0.8	0.001	0.21
96	0.06	0.76	0.001	0.21
104	0.006	0.26	0.002	0.21
120	0.005	0.25	0.001	0.21
128	0.0047	0.24	0.001	0.21
144	-	-		
152	0.0045	0.24		
168	0.006	0.26		
176	0.07	0.39		
192	0.087	0.83		
200				

\* values are means of two successive fermentation.

Table 6 Changes in available protein (%) during the fermentation of 'awaze' and 'datta'.

Time (h)	'awaze'		'datta'	
	Aldehyde value	% protein*	Aldehyde value	% Protein*
0	1.0	0.17	1.5	0.25
8	1.0	0.17	1.5	0.25
24	1.0	0.17	1.5	0.25
32	0.24	0.16	1.5	0.25
48	0.24	0.16	1.5	0.25
56	0.24	0.16	1.5	0.25
72	0.24	0.16	1.5	0.25
80	0.29	0.16	1.4	0.23
96	0.94	0.16	1.4	0.23
104	0.94	0.16	1.4	0.23
120	1.0	0.17	1.4	0.23
128	1.0	0.17	1.4	0.23
144	0.94	0.16	1.4	0.23
152	0.94	0.16	1.4	0.23
168	0.94	0.16	1.4	0.23
176				

\* Values are means of two successive fermentations

## 5 Discussion

Lactic acid bacteria were the most important microorganisms isolated from the fermentation of 'awaze' and 'datta'. Initially the ingredient mixture used to prepare 'awaze' contained both *Bacillus* sp. and lactic acid bacteria while other groups of microorganisms were absent. The absence of such micro-organisms as enterobacteriaceae, coliforms, and staphylococci in the ingredient mixture could be attributed to the intense processing of the raw materials. The absence of other groups of microorganism in the ingredient mixture could also be due to the possible antimicrobial effects of the various spices employed in the mixture, particularly cinnamon and cloves, which are believed to be inhibitory to bacteria at low concentrations (Hersom and Hulland, 1969). The heating process of the spices and the subsequent processing might be responsible for eliminating fungi, and for restricting the types of isolated bacteria. The majority of the added spices such as cardamom, caraway, ginger and the like have little or no antimicrobial effect on yeasts (Herson and Hulland, 1969). The ingredient mixture contained the resistant aerobic bacterial spores and lactic acid bacteria which, among the gram positives, are the most resistant against spices (Zaika et.al., 1983).

The microorganisms involved in the fermentation of both 'awaze' and 'datta' are dominated by lactic acid bacteria although *Bacillus* species were isolated all

through the fermentation. Yeasts also gained prominence in the later stage of the fermentation of 'awaze', which, of course, were not detected originally in the raw materials. In both condiments the initial phase of the fermentation is performed solely by lactic acid bacteria whose numbers increased steadily and reached counts as high as  $10^{11}$  cfu/g for 'awaze' and  $10^9$  cfu/g for 'datta'.

Although many *Bacillus* sp. are involved in the fermentation of several African condiments such as iru and okepeye (Obeta and Ugwuany,1996), they were present in 'awaze' and 'datta' without actually being involved in the fermentation. This is because *Bacillus* spp. do not thrive well in acidic conditions ( $\text{pH}<5$ ) and the count observed in this study was possibility that of dormant but resistant spores. *Bacillus* spp. are, thus, usually important in non-acidic fermentation of a proteolytic type. This finding is, therefore, different from the results obtained by the fermentation of many African plant proteins (Obeta and Ugwuany 1996;Ogbadu and Okagbuye, 1990; Achi, 1992 and Isu and Njoku, 1997) in which *Bacillus* spp. were very important with their numbers increasing throughout the fermentation.

The fact that 'awaze' and 'datta' fermentations are carbohydrate fermentations can be explained by the rapidly increasing and proliferating lactic acid bacteria in the fermenting products. This is in agreement with the report of Jay(1996) that lactic acid bacteria are only weakly proteolytic and lipolytic. Mogessie and

Tetemke (1995) also reported that in the presence of sufficient number of lactic acid bacteria, *Bacillus* species may not contribute in the fermentation of some traditional Ethiopian beverages. The source of the *Bacillus* species which survived the fermentation was the ingredient which consisted of a variety of spices. Spices harbour many species of aerobic spore formers (Palumbo *et.al.*1975). The survival of spores during the fermentation may be due to their resistance to the low pH and high titratable.

The rapid fall in pH and increase in titratable acidity that occurred in the fermentation of both condiments was indicative of the role of lactic acid bacteria in initiating and carrying out the fermentation. Lactic acid levels changed during the process from 0.14%-0.38% for 'awaze' and from 0.04%-0.14% for 'datta' signifying the role of lactic acid bacteria. These acids and other metabolites produced by lactic acid bacteria may contribute to the flavor and texture of the products.

Oyeyiola (1991) reported that spices result in a more acidic product. Thus the relatively lower pH at the initiation of the fermentation might be due to the presence of spices in the ingredients. This could have created a favourable condition for lactic acid bacteria to be active in the initiation of the fermentation and in the production of an acidic product within 48 hours.

Improvement in the flavor of '*awaze*' became evident between 32-56 hour when hetero-lactic fermentation was at its best . Although not investigated in this study heterofermentative lactic acid bacteria impart flavor to products by also producing small amounts of acetic, caproic, valeric and isovaleric acids (Umata and Faulks, 1997). The hetero-lactic activity could also be visually detected due to the continuous bubbling of the fermenting '*awaze*' after 32 hour until the homo lactics took over and gas production ceased.

Even though lactic acid bacteria gained prominence in '*awaze*' fermentation, yeasts were detected, at the later stages (152 h) and their numbers increased markedly from  $10^3$  cfu/g to  $10^6$  cfu/g. This indicated that the later stages of '*awaze*' fermentation was carried out both by lactic acid bacteria and yeasts because the yeasts appeared before the end of the fermentation. This is in agreement with the common occurrence, in food and beverage fermentation, of yeasts and lactic acid bacteria. The lactic acid bacteria provide the acidic environment for yeast growth while the yeasts providing vitamins and other growth factors (Adegoke and Babalola,1988; Oye yiowola, 1991).

The microbial dynamics in "*datta*" fermentation was slightly different from that of '*awaze*'. The presence of such bacteria as enterobacteriaceae, coliforms, staphylococci, and micrococci at the initial stages of fermentation might be due to the fact that '*datta*', unlike '*awaze*', was prepared from fresh vegetables

and spice mixture which had sufficient moisture and which were not heat-treated. It is a common occurrence that growing vegetables are in intimate contact with air, soil and moisture. As a result they harbour a wide range of Gram-positive and Gram-negative aerobic species (Pederson, 1979). The presence of the Gram negative coliforms and enterobacteriaceae which were absent in '*awaze*' may also be due to difference in some intrinsic factors between the two condiments. '*datta*' is mainly prepared from fresh vegetable and spices and most fresh foods have a high water activity (0.99) suitable for the growth of many Gram negative species (Jay,1996).

Even though both coliforms and other enterobacteriaceae were detected at the initial stages of '*datta*' fermentation, they appeared to present no health hazard (Splittstosser, 1980). The role of these coliforms and enterobacteriaceae at the initial stages of '*datta*' fermentation is not known . Steinkraus (1983b) reported the involvement of some members of enterobacteriaceae in the synthesis of vitamins and others are reported to be involved in the fermentation of African Maize-beverages (Akinrele, 1970). The general build up of staphylococci throughout '*datta*' fermentation might have resulted from the wet milling step which involves a frequent contact of the materials with hands.

As the pH fell down and titratable acidity increased all the Gram negative rods were totally inhibited at 48 hour due to the antimicrobial effect of lactic acid

and the depression of pH below the growth range . This is an indication that these Gram negative rods are unable to proliferate in the presence of the lactic groups.

The microbial safety of 'awaze' and 'datta' fermentation tested against a common food-borne pathogen, *Salmonella typhimurium*, revealed that these products have bacteriostatic and bactericidal properties. The test organism was not detected after 56 hour in fermenting 'awaze'. In fermenting 'datta' the test organism was not detectable at 32 hour. As these condiments are not immediately consumed and are left to ferment, the occurrence of the test strain at the initial stage of the fermentation might not be considered as a health hazard. In natural contamination, the initial count of *Salmonella* would be much lower than what was employed in this study. In this study, *Salmonella* introduced at levels of about  $10^3$  cfu/g was not detectable even at 8 hours of fermentation (data not given). A similar test, conducted on Ethiopian traditional fermented milk (Mogessie, 1993) using *Salmonella enteritidis* and *Salmonella typhimurium*, showed that the test strains were not inhibited at 24 hour as in 'datta'. The irregularities in pH and titratable acidity at which this food borne pathogen was inhibited in 'awaze' and 'datta' (because these were different for the two condiments) may suggest that the potential of lactic acid bacteria as bacteriostatic and bactericidal agent in 'datta' could also be due to factors other than pH and acids. Olasupo *et.al.* (1997) isolated bacteriocin

producing lactic acid bacteria from some Nigerian fermented foods which inhibited some food born pathogens including *Salmonella*. Bacteriocin producing *Lactobacillus* which inhibit the growth of other opportunistic food pathogens was also isolated from fermented dry sausages (Tichazek, 1994).

Changes associated with some biochemical properties during the fermentation process are largely influenced by the growth of the microbial populations and their extra cellular enzymes. The most important biochemical change during 'awaze' and 'datta' fermentation is carbohydrate hydrolysis which was particularly significant for 'awaze' fermentation. The initial increase in the reducing sugar level may be due to the enzymatic breakdown of starch by the microbial amylase. Throughout the fermentation, *Bacillus* spp. have been isolated. The presence of these organisms has an important bearing at the initial stage of the fermentation as their extracellular amylase breaks down available starch. It is, hence, believed that breakdown of the starch substrate stimulates the growth of lactic acid bacteria (Achi, 1990). The subsequent decrease in reducing sugar level between 32-48 hrs. may be due to the utilization of the sugar by the proliferating lactic acid bacteria. This is in agreement with the fermentation of a variety of vegetables (Odunfa, 1985b)

The initial rise in the level of reducing sugar for 'datta' can also be due to the microbial hydrolysis of available starch by amylolytic *Bacillus* spp. But here

the fluctuation was very little and after a small decline in the sugar level, it was found to stabilize. This may be due to the relatively small count of lactic acid bacteria in '*datta*', so that not much of the sugar was converted to lactic acid. The pH drop during the whole process was less than 1 unit.

In both '*awaze*' and '*datta*' there was very little change in the protein content through out the fermentation period. This is really due to the fact that the vegetables used in the study were not good sources of protein. A majority of African Condiments are produced by vegetable protein fermentations (Odunfa, 1985 b and Ogbadu et.al. 1990) accompanied by a rapid rise in pH to an alkaline reaction. The rise in pH in such vegetable protein fermentations due to the production of ammonia (Ogbadu and Okagbue, 1988) by protease and deaminase enzymes of the fermenting micro flora is not in agreement with the results of this study. In this case the pH of both '*awaze*' and '*datta*' showed a marked decline with no significant change in the protein content of the fermenting material.

## 6 CONCLUSION

These two Ethiopian condiments, 'awaze' and 'datta' are fermented by Lactic acid bacteria which add a distinctive flavor and aroma to the products by their ability to produce a variety of organic acids and other metabolites. The involvement of yeasts at the later stage of 'awaze' fermentation did not seem to influence flavor and aroma development as these characteristics were prominent even before the appearance of yeasts. However the yeasts, as their number increased during the process, might be involved in the synthesis of certain vitamins useful for the lactic acid bacteria.

The absence of gram-negative rods such as members of enterobacteriaceae and the coliforms in 'awaze' and their disappearance at the later hours of 'datta' fermentation indicates the microbial safety of these condiments from certain food borne pathogens such as *Salmonella*.

Both 'awaze' and 'datta' are entirely carbohydrate fermentations indicated by the proliferating homo and heterolactics which resulted in a drop in pH all along the fermentation, and also a change in reducing sugar. Thus these lactic acid bacteria which added flavor and aroma to the products should be further characterized and used in further studies on controlled fermentation and process optimization.

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