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Research Trends in Modern Food Fermentation Biotechnology and Ethiopian Indigenous Traditional Fermented Foods and Beverages Research Achievements: A Review

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Abstract

Biotechnology is as old as human civilization and has been an integral part of the human life and is believed to originate more recently to its modern form from an indigenous knowledge of African traditional food and beverage fermentation practices. The application of modern biotechnology methods in the food and agricultural industry is expected to alleviate hunger today and help avoid mass starvation in the future. Nowadays, modern food biotechnology has been transforming our traditional way of food production and preparation far beyond the traditional scope. Currently, at global level, food biotechnological research has focused on; traditional process optimization (starter culture development, enzymology), food safety and quality, nutritional quality improvement and food preservation (improving shelf life). Special emphasis has also been given to the newly growing concept such as functional foods and probiotics. To this regard, the science has gone more beyond our expectation and exemplary achievements are described here. In this review, food biotechnological research on Ethiopian indigenous traditional fermented foods and beverages was assessed in view of this global achievement. Microbiological study on Ethiopian traditional fermented foods and beverages has focused more on Isolation, identification, characterization of fermentative microbes, attempts to develop starter culture, physicochemical, biochemical, nutritional composition change during fermentation, depicting general traditional processing and recently on probiotics. Most of the researches have been concentrated on some very commonly and widely known traditional fermented foods and beverages nearly around the central and southern part of the country. The research was also fragmented and not progressive for a given food and/or beverage. Biotechnological researches on Ethiopian traditional fermented foods and beverages have not gone far beyond some attempts on molecular characterization study of fermentative microorganisms. The reasons for this limitation could be linked to the fact that; biotechnological research is capital intensive, requires the use of sophisticated equipment, reagents, chemicals and needs sufficient trained man power in the field. Lack of funding, little attention from government policy and the endemicity of the traditional fermentation techniques may also have contributed for the less/no advancement of the techniques. In conclusion, to promote Ethiopian indigenous traditional fermented foods and beverages into commercialization/ industrialization, using currently advancing food fermentation biotechnology, we need to: realize the economic benefits of these indigenous traditional knowledge, develop our human, technical and institutional capability, go out to assess the cultural and geographical diversities of the country, broaden our research topics far beyond the current, and keep on continuous and progressive research.

Keywords: Fermentation, Biotechnology, Traditional Fermented Foods, Beverages

Introduction

Biotechnology is as old as human civilization and has been an integral part of the human life. Food and beverage fermentation is regarded as one of the oldest ways of food processing and preservation; and this knowledge has been an "indigenous knowledge" to all parts of the world (Steinkraus, 2002). This pioneering indigenous knowledge of food and beverage fermentation practice has been considered to be the first biotechnology originated in developing countries (Anon, 1992). Hamid (1992), argued the fact that this technology has originated in Africa by saying "If we accept the idea that Africa is the birthplace of Man, it would seem logical that the first human or humanoid to consume a fermented food would have lived there". Many scientists use the term old or traditional biotechnology to these natural processes that have been used since many centuries to produce fermented foods and beverages. The new or modern biotechnology started more recently and is rapidly growing. It includes all the genetic manipulations, cell fusion techniques and the improvements made in the old biotechnological processes. According to the Convention on Biological Diversity (CBD, 2000), biotechnology is defined as "any technological application that uses biological systems, living organisms or derivatives thereof, to make or modify products or processes for specific use". Another definition still describes biotechnology as the application of scientific and engineering principles to the processing of materials for the provision of goods and services through the use of biological systems and agents (Anon, 1992). International food science and technology (IFST, 2008), specifically defined food biotechnology as "the application of biological techniques to food crops, animals and microorganisms to improve the quality, quantity, safety, ease of processing and production economics of food". Since its invention, modern biotechnology has been playing a great role in human life. However, though Africa has been inferred the origin of biotechnology, these village-art methods and age-old techniques are still used in many developing countries like Ethiopia. This paper briefly summarizes some of the current research advances in food fermentation biotechnology and research achievements on Ethiopian indigenous traditional fermented foods and beverages in view of the recent food biotechnology research. However, this article is not a documentation of every work done, but is a comprehensive review.

Current research trends in modern food fermentation biotechnology: A brief highlight

Modern biotechnology enables plants, animals and microorganisms to be genetically modified (GM) with the inclusion of needed novel traits. The inclusion of the desired novel traits potentially offers improved quality, nutrition and processing characteristics, which can allow the production of more nutritious, safer, tastier, and healthier food. Techniques in biotechnology, particularly advances in recombinant technology have been transforming our traditional way of food production and preparation by improving the foods we eat and the beverages we drink far beyond the traditional scope. Currently, the transformation has focused more on traits which improve, nutritional quality, sensory quality, bacteriophage resistance, ability to produce antimicrobial compounds such as bacteriocins, degradation or inactivation of natural toxins such as cyanogenic glucosides, mycotoxins, anti-nutritional factors such as phytates, elimination of carcinogenic compounds such as ethylcarbamate in alcoholic beverages. Another special focus is on enzymes, the biological catalysts used to facilitate and speed up metabolic reactions in living organisms. Some advanced biotechnological research examples are highlighted.

Cyanogenic glycosides in Cassava

Cassava (*Manihot esculenta* Crantz) is widely used throughout Africa as an important food source (Oyewole, 1995) but its major problem is that it contains poisonous compounds called cyanogenic glycosides, linamarin and lotaustralin which liberate cyanide (CCDN, 2010).The hydrolysis of cyanogenic glucosides by the endogenous or microbial linamarase enzyme releases the hydrocyanic acid (HCN), which is toxic. Cyanogenic glucosides are thus responsible for the toxicity of unfermented roots and leaves of cassava (CCDN, 2010) and yet, the total cyanide content of cassava fermentation in Ethiopia was determined to be 1.51-2.81 mg HCN/100 g (Enidiok *et al*., 2008). Unhydrolysed linamarine, remaining in cassava roots and leaves after fermentation, can constitute a health problem for the consumers and is lethal at a consumption dose of 0.5 to 3.5 mg per kilogram body weight (Gomez and Valdivieso, 1985). Konzo is a paralytic disease (with identical clinical symptoms to Neurolathyrism) associated with consumption of insufficiently processed cassava and cases are found in Sub Saharan Africa (CCDN, 2010).Traditionally, most of these constraints have been met through processing such as boiling, smoking, drying and fermentation, though fermentation is by far the most important and widely used means of cassava processing (Oyewole, 1992). However, these traditional practices are overwhelmed with so many problems for which modern biotechnology offers the best solution. For example, improvements in cassava processing would help to maximize the detoxification process.

Biotechnology has been identified as a scientific tool that could be used to meet the current challenges in the traditional fermentation processing of cassava. Biotechnological research on cassava fermentation developed appropriate starter culture that can produce amylase and linamarase enzymes necessary for starch breakdown and cyanogenic glucoside hydrolysis, respectively. Strains of *Lactobacillus plantarum* were found to produce these enzymes. The amylase and linamarase enzymes produced by one of the *Lactobacillus plantarum* strains *(GL 721)* were purified and characterized for their pH and temperature optimal activity (Oyewole, 1995). According to Oyewole (1992), to initiate genetic manipulation of cassava lactic acid bacteria, the plasmid profiles of the lactobacilli isolated from cassava were studied and the presence of plasmids has been confirmed and further biotechnological research approach has been given hope for investigating improvement of the process.

Ethylcarbamate in alcoholic beverages

Ethylcarbamate or urethane is one of the undesirable by-products formed during yeast fermentation of foods and beverages and it is a potential carcinogenic substance (Mattew *et al.,* 2009). Recombinant DNA technologies has made possible to introduce new properties into the yeast, as well as to eliminate the undesirable by-products. Ethylcarbamate is synthesized by the spontaneous reaction between ethanol and urea (Fig. 1) (Joana *et al.,* 2006). Yeasts used in wine fermentation possess arginase enzyme that catalyzes degradation of arginine (found in large amount in grapes) into urea.

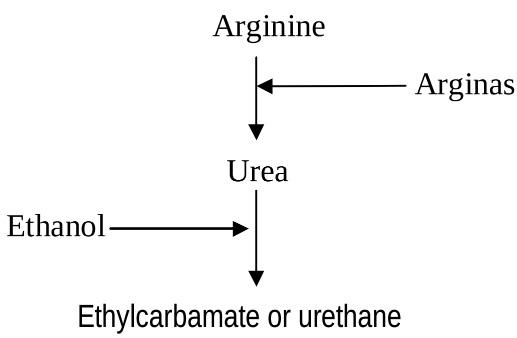

Figure 1. Simple simulated presentation of Ethylcarbamate formation

Basic thoughts that led to the investigation

If this enzyme can be blocked, arginine will no longer be degraded into urea and hence, no urea to react with ethanol to form ethylcarbamate, then, no chance of cancer development.

According to Kitamoto *et al.* (1991), a transgenic yeast strain in which the *CAR1* gene (encodes enzyme arginase) is inactivated was developed to reduce the formation of urea. As a result, urea was eliminated and ethylcarbamate was no longer formed during fermentation.

Acrylamide in foods

Acrylamide is a suspected carcinogenic and toxic molecule, whose presence in foods was first detected in 2002 (CAST, 2006). A wide range of cooked foods contain acrylamide at different amount (Otles and Otles, 2004). Anese *et al.,* (2009) has summarized mechanisms of acrylamide formation and the most important measures regarding acrylamide mitigation including food fermentation. However, use of biotechnological tools is one and considered most promising. As a result of this biotechnological tools, an enzyme asparaginase from cloned *Aspergillus oryzae* has been developed and is claimed to reduce acrylamide levels by up to 90% through the conversion of asparagine into aspartic acid (IUFoST, 2010) without altering the appearance or taste of the final product.

Enzymes, amino acids, vitamins, bioflavors and carotenoids

Nowadays, many enzymes used in food processing industries are derived through the use of genetically modified microorganisms (GMMs). Genetically modified yeasts, fungi and bacteria have been in commercial use for this purpose for over a decade (Ray, 2004). Examples include: chymosin for cheese-making. Chymosin, commonly known as rennin, is the principal milk-coagulating enzyme present in rennet. Currently, recombinant DNA technology has made it possible to obtain chymosin from strains of microorganisms. The gene for chymosin has been cloned and inserted into *Escherichia coli* K-12, *Kluyveromyces lactis, Aspergillus niger* var. *awamori* resulting in the expression for use as a coagulant (Fox and Law, 1991). Chymosin produced by these recombinant microorganisms is currently commercially produced and is widely used in cheese manufacture. Many food-processing enzymes produced through the use of GMMs have been on the market for over a decade, and are used in a wide variety of processed foods (IUFoST, 2010).

Genetically modified microorganisms have also been in use for the production of various substances such as micronutrients (vitamins, amino acids), bioflavors, and carotenoids (Ray, 2004). As an example, researchers isolated a xylose reductase (*XYL1*) gene from *Candida shehatae* and introduced it into *Saccharomyces cerevisiae*. The genetically transformed *S. cerevisiae* has been used for commercial production of xylitol from xylose (Govinden *et al.* 2001). Biotechnological studies also genetically transformed the edible yeast *Candida utilis by using genes from Erwinia uredovora* and *Agrobacterium aurantiacum* and used the resultant transgenic *C. utilis* as a potential microorganism for large-scale production of commercially important carotenoids (Miura *et al.*,1998).

Most of the microorganisms modified for food processing are derivatives of microorganisms used in conventional food biotechnology. Microorganisms improved by modern biotechnology are also under development in the field of probiotics (WHO, 2005). According to FAO/WHO (2001), probiotics are "live microorganisms, which, when administered in adequate amount, confer a health benefit on the host". Probiotics have got momentum for their health benefits as anti-cholesterol, anti-cancer, immunoactive effects, anti-diarrhea effects (Kailasapathy and Chin, 2000) and more. Nowadays, probiotic foods receive market interest as health-promoting functional foods (foods or dietary components that may provide a health benefit beyond basic nutrition) and tremendous lists are available commercially (Fig. 2) in many developed countries (WGO, 2008).

Fig. 2 Commercially produced and available probiotic bacteria
Source: http://microbewiki.kenyon.edu/index.php/File:Lplantarum.jpg (Left).
Mercola.com "The Newfound Link Between Probiotics and Your Weight" (right)

Research on Ethiopian Indigenous traditional fermented foods and beverages

Ethiopia is endowed with ethnic diversity manifested in cultural diversity and a variety of traditional fermented foods and beverages. According to Mogessie (2006), microbiological study on Ethiopian traditional fermented foods and beverages has started in the 1980s on topics like microbial succession and accompanying changes, food safety, processing and spoilage. Most of these works were concentrated on some very commonly and widely known traditional fermented foods and beverages nearly around the central and southern part of the country. Since then, however, the works have been getting momentum in research topics, geographical (ethnic group), traditional fermented foods and beverages diversities (Table 1 & 2), though it is not yet to the level needed. For example, detailed works on the microbiological study of *wakalim*, a traditional fermented beef sausage among the Harari people was the first of its kind (Ketema *et al.,* 2007). Fermentation yeast characterization study on indigenous fermented honey wine, known locally as *ogol* from Majangir ethnic group in Gambella region was another example of such diversification (Teramoto *et al.*, 2005). Characterization of probiotic bacteria (Ketema *et al.,* 2009; Asnake and Mogessie, 2010) and study on technological modifications (Kebede *et al.,* 2004) of Ethiopian traditional fermented foods and beverages has also indicated encouraging progress.

However, in light of current global advances in food fermentation biotechnology and commercial-ization / industrialization, research on Ethiopian indigenous traditional fermented foods and beverages are not yet approaching to the expectations. If we are in a position to accept, the idea of African indigenous knowledge of food and beverage fermentation as the first biotechnology (Anon, 1992); Africa as the birthplace of Man and the African to be the first to consume a fermented food (Hamid, 1992), the lion share of this idea should be taken by Ethiopia.

Table 1. Studied Ethiopian indigenous traditional fermented foods and research topics emphasized

No.	Traditionally Fermented Food	Research works	Microbes suggested to be involved in the fermentation	References
I	**Animal origin**			
1	**Dairy products** ***Ergo*** (sour milk) ***Ititu*** (concentrated sour milk-prepared and consumed by Borena tribe) ***Ayib*** (cottage cheese) ***Qibe*** (traditional butter) **Arrera** (defatted butter milk)	Isolation, characterization, identification of fermentative microbes Starter culture evaluation Physicochemical and biochemical change determination Determination of nutritional composition change during fermentation General flow scheme for traditional processing	Lactic acid bacteria	Mogessie, 2006; Almaz *et al.*, 1999; Fekadu *et al*, 1998
2	***Wakalim*** (Ethiopian traditional fermented beef sausage)	Isolation, characterization, identification of fermentative microbes Probiotic bacteria characterization General flow scheme for traditional processing	Lactic acid bacteria	Ketema *et al.*, 2010; Ketema *et al.*, 2009; Ketema *et al.*, 2007
II	**Plant origin**			
1	***Enjerra* (tef dough)**	Isolation, characterization, identification of fermentative microbes Physicochemical and biochemical change determination Determination of nutritional, anti-nutritional factors composition change during fermentation Probiotic bacteria characterization	Enterobacteriaceae *Bacillus* spp. Lactic acid bacteria Yeast	Asnake & Mogessie, 2010; Mogessie, 2006; Ayele *et al.*, 1997; Kelbesssa, *et al.*,1997a; Berhanu, 1982; Chaltu & Abraham, 1982

2	***Kocho***	Isolation, characterization, identification of fermentative microbes Physicochemical and biochemical change determination Determination of nutritional composition change during fermentation General flow scheme for traditional processing	Lactic acid bacteria Yeasts *Bacillus* spp.	Tariku & Mogessie, 2011; Mogessie, 2006; Ayele, 2000; Kelbesssa, *et al.,* 1997b; Berhanu , 1987
3	***Siljo* (condiment)**	Isolation, characterization, identification of fermentative microbes Physicochemical and biochemical change determination Determination of nutritional composition change during fermentation General flow scheme for traditional processing	Lactic acid bacteria Yeasts *Enterococcus* *Bacillus* spp.	Mogessie, 2006; Senait, *et al.,* 1995; Tetemke & Mogessie, 1995
3	***Awaze* (condiment)**	Isolation, characterization, identification of fermentative microbes Physicochemical and biochemical change determination Determination of nutritional composition change during fermentation Probiotic bacteria characterization	Lactic acid bacteria Yeasts	Asnake & Mogessie, 2010; Mogessie, 2006; Ahmed, *et al.,* 2001
5	***Datta (qotchqotcha ?)* (condiment)**	Isolation, characterization, identification of fermentative microbes Physicochemical and biochemical change determination Determination of nutritional composition change during fermentation Probiotic bacteria characterization	Lactic acid bacteria	Asnake & Mogessie, 2010; Mogessie, 2006; Ahmed, *et al.,* 2001

Table 2. Studied Ethiopian indigenous traditional fermented beverages and research topics emphasized

No.	Traditional Fermented Beverages	Research works	Microbes suggested to be involved in the fermentation	References
I	**Plant origin**			
1	***Tella***	Isolation, characterization, identification of fermentative microbes Physicochemical and biochemical change determination Determination of nutritional composition change during fermentation	Lactic acid bacteria yeast	Mogessie, 2006; Samuel & Berhanu, 1991
2	***Tej* (honey wine)** **(*Ogol*** *in Gambella***)**	Isolation, characterization, identification of fermentative microbes Physicochemical and biochemical change determination Determination of nutritional composition change during fermentation General flow scheme for traditional processing	Lactic acid bacteria yeasts	Bekele, *et al.*, 2006; Mogessie, 2006; Teramoto *et al,* 2005;
3	***Borde***	Isolation, characterization, identification of fermentative microbes Physicochemical and biochemical change determination Determination of nutritional composition change during fermentation General flow scheme for traditional processing	Lactic acid bacteria yeasts	Mogessie, 2006; Kebede, *et al.,* 2002; Ketema, *et al.,* 1998; Mogessie & Tetemke, 1995
3	***Shamita***	Isolation, characterization, identification of fermentative microbes Physicochemical and biochemical change determination Determination of nutritional composition change during fermentation General flow chart of *Shamita* produced in laboratory	Lactic acid bacteria yeasts	Mogessie, 2006; Ketema *et al.,* 1999; Mogessie & Tetemke, 1995

However, though Ethiopia has been inferred the birth place of Man and the origin of biotechnology, biotechnological researches on Ethiopian traditional fermented foods and beverages have not gone far beyond some attempts on molecular characterization study of fermentative microorganisms (Ayele, 2000). Okafor (1992) measured the extent of commercialization of some important fermented foods of sub-Saharan Africa on a scale of eight. Very few Ethiopian fermented foods included in his review were categorized at a stage of scale one and two, which means that the works done focused mostly on isolation and determination of role(s) of organisms involved in the food, respectively.

The reasons for this limitation could be linked to the fact that biotechnological research is: capital intensive usually in scarce foreign exchange, requires the use of sophisticated equipment and reagents backed with a consistent energy and water supply, needs purchase of essential chemicals which cannot be obtained in months or even years (Anon, 1992). Lack of sufficient trained man power in that specific field of research, lack of funding for such research activities, and negligence from government policy for commercialization/industry-alization of indigenous traditional fermented foods and beverages might have also contributed a lot. Traditional fermentation techniques limited to a particular country are less likely to advance in technologies because of less research effort from various workers, and Ethiopian traditional food and beverage fermentation technology being limited to the country alone may also account for less advancement of the technology.

Lesson and gaps

A new development in biotechnology in general and food biotechnology in particular has been accelerating the scope of food related researches into a wider scale up application. As a result of advanced research on traditional fermented foods and beverages, various other African traditional fermented foods and beverages have been transformed from traditional processing technology into semi-industrial and industrial level production technology. A good example is a Nigerian condiment known as *dawadawa,* produced under the trade name of Dadawa from African locust beans *(Parkia biglobosa)* seeds. The rate of transformation of this condiment was rapid and interesting. In 1981 the organisms involved were unknown; in 1991 not only are they known, but the food itself has been commercialized (Okafor, 1992). Cadbury Nigeria PLC now markets the traditional fermented seeds of *Parkia* under this trade name, Dadawa (Achi, 2005). South African sorghum beer (*kaffir)* is another good example for traditional fermented beverages modified for industrial production and which is now being sold in South Africa packed in milk cartons (Achi, 2005). A range of traditional fermented foods and beverages transformed/modified into semi-industrial and industrial scale production has been well summarized (Okafor, 1992; Achi, 2005).

Figure 3. Konso people drinking traditional fermented beverage along with the fermentative microbes (Photo source: http://www.pbase.com/sergio_pes/konso_people)

Now we can think of about varieties of Ethiopian indigenous traditional fermented foods and beverages being produced and sold by traditional producers, and others which even their existence have not been yet known. Considering the diversity of ethnic groups of Ethiopia, there is no doubt that there will be lots of indigenous traditional fermented foods and beverages yet to be studied. A quite number of works done so far have concentrated on such indicated works (Table 1 & 2), and were also fragmented and not progressive for a given food and/or beverage. As far as my literature search is concerned, there has been no work done towards optimization/commercialization/industrialization of Ethiopian indigenous traditional fermented foods and beverages. A reader can ask a question like, why a traditional fermented food and/ or beverage called 'X' has not been yet studied, modified/industrialized?

The way forward

The application of biotechnology methods in the food and agricultural industry is one of the many aspects of biotechnology that has great impact on society. By the year 2020, Africa's population is expected to double, and that Africa's high population growth rate means feeding an additional 20 million people per year as well as provides them with other basic needs (UNECA, 2000). Biotechnology is expected as a scientific field that offers the greatest potential to stop hunger today and help avoid mass starvation in the future (Jube and Borthakur, 2006). According to Anon (1992), to meet the current and future challenges in developing countries, it is important that these countries develop the capabilities to benefit from biotechnological developments, acquire expertise in biotechnology through education and training, and establish infrastructure and equipment required for biotechnological research. Recognizing the benefit that the safe application of modern biotechnology offers for its agricultural development, Ethiopia has put forward favorable policies to facilitate its use (Adane, 2009), though the emphasis has been more on production/yield increase than on food processing technologies. In this case contributions of food processing technologies/food fermentation biotechnologies for the national economy should not be overlooked. Many countries have benefited a lot from commercialization/industrialization of their indigenous traditional fermented foods and beverages both in value addition to the raw materials (agricultural produces) and from sale of final processed products. For example, in Indonesia about 76, 000 tons of soybeans are used annually in the fermentation of tempe and about 128, 000 workers are employed in the tempe industry (Achi, 2005). Here, we can imagine how many of Ethiopian has been self employed as traditional producer of fermented foods and beverages and get their daily income from the sale without any intervention from scientific research. Ethiopian traditional fermented foods and beverages, with no doubt are good functional foods and rich in various probiotics. The traditional fermented foods and beverages such as *Awaze*, *Datta (qotchqotcha), Borde* and *Shamita* are definitely rich in probiotics and hence confer the health benefits (Fig. 3). So, why do not we search for these potential and accelerate the economic use?

To make best use out of these indigenous traditional fermented foods and beverages we need to emphasis on our manpower, technical, institutional and policy constraints. For example, in Ethiopia there is no government organization mainly working on promoting indigenous traditional food and beverage fermentation practices toward modern food processing technology or even documenting such cultural heritage available in the country. The agricultural production sector policy of the country has mostly focused on production/yield increase. External fund providers also focused more on production/yield increase. This approach is a wonderful approach for countries which have not yet attained self food sufficiency. However, parallel considerations to promote (commercialize/industryalize) such indigenous technologies also contribute to the country's economy in many ways. For example, it provides linkages between agricultural producers and processors creating good opportunity for value addition on agricultural raw materials (produces) boosting individuals' economy in particular and the country's in general.

In conclusion, to promote Ethiopian indigenous traditional fermented foods and beverages into commercialization/industrialization, using currently advancing food fermentation biotechnology, we need to: (1) realize the economic benefits of these indigenous traditional knowledge; (2) developing human, technical and institutional capability; (3) go out to assess the cultural and geographical diversities of the country; (4) broaden our current research topics towards optimization/commercialization/industrialization; and (5) keep on continuous and progressive research activities towards the needed scientific level.

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