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PROBIOTIC POTENTIAL AND NUTRITIONAL IMPORTANCE OF TEFF *(Eragrostis tef (Zucc) Trotter)* ***ENJERRA*** - A review

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ABSTRACT

Fermentation of cereals for a limited time improves amino acid composition and vitamin content, increases protein and starch availabilities, and lowers the levels of antinutrients. Although cereals are deficient in some basic components (example, essential amino acids), fermentation may be the most simple and economical way of improving their nutritional value, sensory properties, and functional qualities. Functional foods have nutritional and physiological benefits and are applicable in disease prevention and management. Enjerra (*Injera*) is the national food of Ethiopians. It can be made from different cereals, including sorghum, teff, corn, finger millet and barley, although teff [Eragrostis tef (Zucc) Trotter] is the major cereal ingredient in Ethiopian *e*njerra. Interest in teff has increased noticeably due to its very attractive nutritional profile and gluten-free nature of the grain, making it a suitable substitute for wheat and other cereals in their food applications as well as foods for people with celiac disease. Because of its small size, teff is made into whole-grain flour (bran and germ included), resulting in a very high fibre content and high nutrient content in general. In traditional approaches of teff fermentation, the advantages of some form of inoculation of a new batch, such as back-slopping or the repeated use of the same container and a liquid starter 'ersho', are appreciated and generally practised. Still, the benefits of starter culture application as a means of improved functionality are not yet realised in teff fermentation operations. Enjerra quality, safety and functionality would be significantly improved through optimization of the traditional fermentation process on the basis of multifunctional considerations, also taking into account the dominant lactic acid bacteria (LAB) and yeasts involved, and possibilities offered for improved health benefits. There is considerably less information with regard to quality and functionality of teff enjerra. In this review, the available literature concerning teff enjerra fermentation process, involved microorganisms' dynamics, the nutritional improvement of teff by fermentation and its potential functional (probiotics and prebiotics) properties have been compiled and are critically analyzed. Future research needs to improve teff enjerra's nutritional and probiotic properties, and the possibilities for industrialization of its production are addressed.

Key words: Fermentation, Lactic acid bacteria, Fibre ajfand SCHOLARLY, PEER REVIEWED AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT Volume 15 No. 2 March 2015 PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST ISSN 1684 5374

INTRODUCTION

The cereal grain teff (*Eragrostis tef [Zucc.] Trotter*) is one of the major cereal crops of Ethiopia, where it is believed to have originated [1]. It is the most popular cereal grain for making *enjerra*, which forms the traditional basic diet in Ethiopia, although other grains such as sorghum, maize, barley, wheat and finger millet are sometimes used [1, 2]. Teff has the largest share of area (23.42%, 2.6 million hectares) under cereal cultivation and third (after maize and wheat) in terms of grain production (18.57%, 29.9 million quintals) in Ethiopia [3]. The principal use of teff grain for human food is the Ethiopian bread *enjerra*, a soft porous thin pancake with a sour taste [4, 5]. *Enjerra* is made from flour, water and starter *ersho* [6]. *Ersho* is a fluid saved from previously fermented dough.

Teff provides over two-thirds of the human nutrition in Ethiopia, with grain protein content (10-12%) similar to other cereals [1]. Teff proteins have non-gluten nature and owing to prevailing portion of prolamins belong to easily digestible ones [5], which make it a suitable alternative to wheat in the case of celiac disease and gluten-free diet. Besides providing protein and calories, it has high nutritional content, including better amino acid composition, especially lysine, more mineral content (mainly iron, calcium, phosphorus and copper) than other cereal grains, contain B1vitamin and is rich in fibre [1, 2, 4-7].

Fermentation is one of the oldest and most economical methods of producing and preserving food [8]. It is found to destroy undesirable components, to enhance the nutritive value, flavour and taste of the food, and to make the product safe from pathogenic microorgansisms [6, 8]. In indigenous fermented foods, the microorganisms responsible for the fermentation are usually the microflora naturally present on the raw substrate [6, 8, 9]. Backslopping, that is, inoculation of the raw substrate with a small quantity of a previously performed successful fermentation, is used to optimise spontaneous fermentation. This kind of a starter, which is a previously fermented product, is used not only to initiate the fermentation but also to accelerate the initial phase of fermentation and keep a uniform quality from batch to another [6, 9, 10].

Foods that, in addition to their basic nutrients, contain biologically active components that can have a positive impact on the health of the consumer are defined as functional foods [11]. Associated with fermentation are useful microorganisms, referred to as probiotics, most of which belong to the genera *Lactobacillus* and *Bifidobacterium* [11]. These health enhancing microorganisms bring about fermentation resulting in production of lactic acid and hence commonly referred to as lactic acid bacteria (LAB) [11, 12]. The term LAB is used to describe a broad group of Gram-positive, catalase-negative, non-sporing rods and cocci, usually non-motile, that utilize carbohydrates fermentatively and form lactic acid as the sole or major end product. Prebiotics, on the other hand, are non-digestible food ingredients that affect the host by selectively targeting the growth and/or activity of one or a limited number of beneficial bacteria in the colon, and thus have the potential to improve health [13]. Cereals are good substrates for the growth of probiotic strains and due to the presence of non-digestible components of the cereal matrix may also serve as prebiotics [14].

Teff *enjerra* is getting popularity in Ethiopia as well as in the developed world because of its being a whole grain product and gluten free nature, the cause for celiac disease [15, 16]. It is high in protein, carbohydrates, and fibre. The protein composition offers an excellent balance among the essential amino acids [16]. However, teff fermentation process has not received the scientific attention it deserves in the last decades with regard to functional properties and health benefits. There is shortage of basic information that could be utilized to advance further researches on teff fermentation process optimization for improved nutritional and health quality of the people in Ethiopia as well as in other countries where teff *enjerra* is used as common food item. Therefore, this paper aims to review *enjerra* production process, dominant bacteria and yeasts involved at different stages, health enhancing properties and, potential pre- and probiotic attributes of teff *enjerra*. Future research needs to improve teff *enjerra* are also addressed.

TEFF *ENJERRA*

The principal use of teff *[Eragrostis tef (Zucc) Trotter]* is in *enjerra* production that constitutes the 70% of Ethiopians diet [16]. Teff *enjerra* is the most common and the main staple food in much of the central, western and northern highlands of Ethiopia as well as among the urban community [1, 2, 6, 17, 18]. Wherever the soil type and rainfall patterns are suitable for cultivation of teff, *enjerra* from teff is more favoured than that from the other cereals [1, 2, 18].

Fermentation Process

The preparation of teff *enjerra* consists of two stages of natural fermentation, which last for about 24 to 72 hours, depending on ambient temperatures [16, 19]. Temperature in the highlands of Ethiopia is generally between 17 and 25ºC [16]. The only required ingredients are the teff flour and water. An appropriate amount of flour is mixed with twice its weight of water [6, 16]. This is kneaded thoroughly to produce a thick paste. Inoculation is accomplished by consistently using partially cleaned fermentation container (backslopping) and by adding some *ersho*, a clear, yellow liquid that accumulates on the surface of the dough towards the final stage of a previous fermentation [6, 9, 17]. About 480 g *ersho* is added to 3 kg teff flour and 6 L of water [16].

The initial 18 hours of fermentation are characterized by vigorous evolution of gas and maximum dough-rising [20]. This is followed by the appearance of an acidic yellowish liquid on the surface of the dough at about 30-33 hours of fermentation [6, 20]. Gas evolution decreases after the pH has fallen below 5.8 (31 hours) [20]. The liquid layer is discarded at the end of the first stage of fermentation. As soon as the liquid layer is poured off, about 10% of the fermenting dough is mixed with three parts of water and boiled for 2 to 5 minutes [6, 16]. This is called *absit*, a dough enhancer, and it is mixed with the rest in the fermentation vat [9, 20]. *Absit* ensures that *enjerra* will have the proper texture and consistency [6, 16, 20] and the dough-rising and gas formation processes are enhanced so they occur in a short time [20]. This process signals the initiation of the second stage of fermentation.

Enjerra baked without *absit* or with less *absit* than the required will have fewer amounts of *eyes* (pits) on the upper surface. A higher number of larger *eyes* are a very desirable attribute of an attractive *enjerra*. *Enjerra* baked at 24 hours or less is called *aflegna enjerra* and has sweet taste [6, 16]. It is recommended for people suffering from gastritis and those who do not tolerate acidic foods [6, 9].

Maximum dough-rising, which normally takes 30 minutes to 2 hours, signals the termination of fermentation [16, 20]. At this stage the fermenting dough is thin enough to pour onto the hot flat pan, locally known as *mitad* for steam-baking into *enjerra*. The total baking time for one *enjerra* is 2 ½ - 3 ½ minutes [6]. *Enjerra* storage period does not usually exceed three days at ambient temperature (temperature in the highlands of Ethiopia is between 17 and 25^{o} C) under the traditional storage conditions essentially due to mould spoilage [6, 20].

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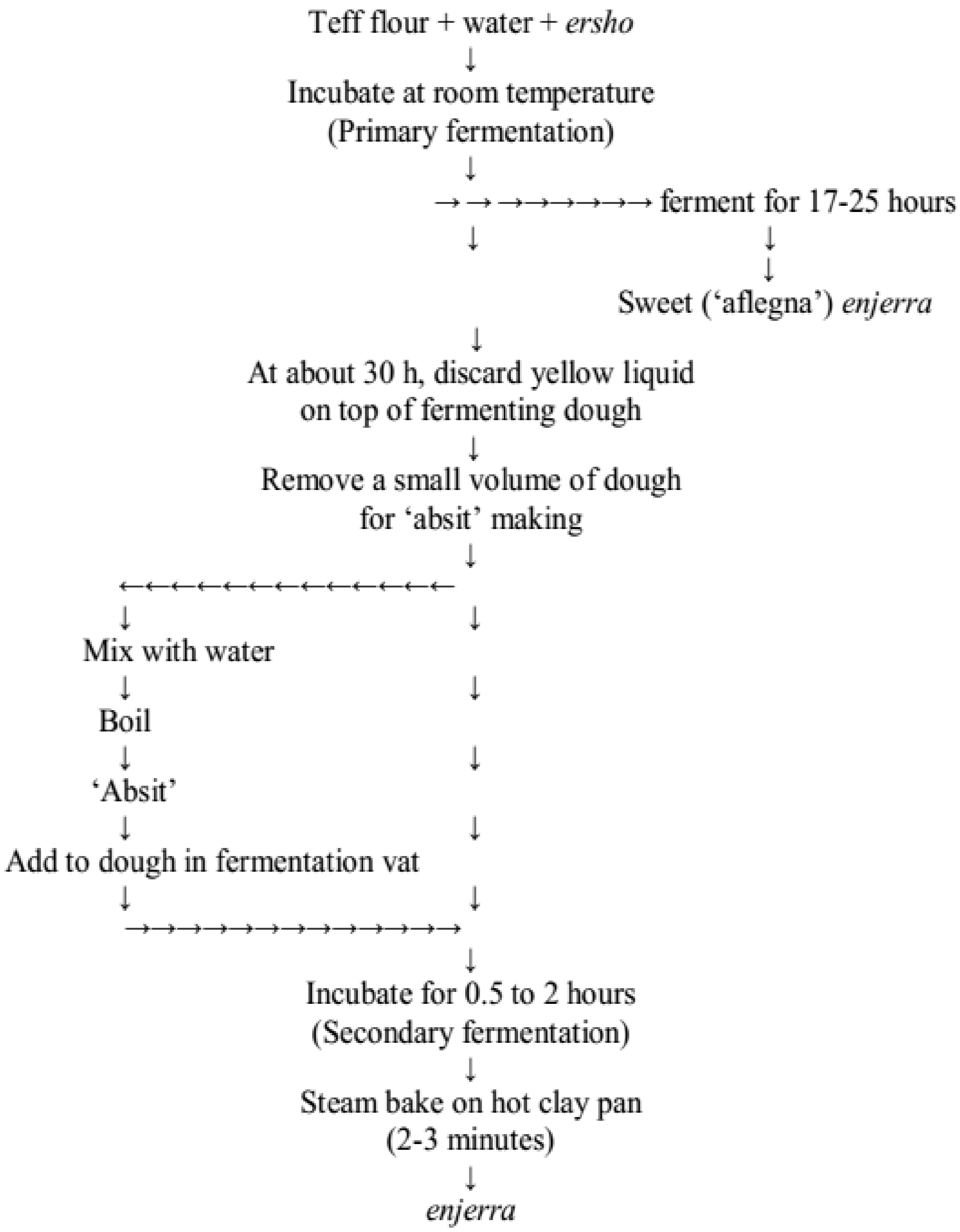

Figure 1: *Enjerra* production flow chart. Adapted from Steinkraus 1983 [19]

Microbiology

The type of microbial flora developed in each fermented food depends on the water activity, pH, salt concentration, temperature and the composition of the food matrix [8]. A primary source of inoculum for teff fermentation is the teff flour itself [6]. The traditional threshing processes of teff would result in the contamination of the teff seeds with a wide variety of microorganisms of soil and fecal origin [21].

Studies conducted on fermentation of teff using it as the sole source of microorganisms indicate that a metabolically associated heterogeneous group of fermentative, aerogenic, gram-negative rods, lactic acid bacteria, *Bacillus spp*. and yeasts growing in succession were involved in the fermentation process of teff [20, 22].

The metabolism of the lactic acid bacteria (LAB) is focused on the effective use of many different carbohydrates, the fermentation of which provides energy (as ATP) for its use in mainly biosynthetic pathways during cell division. Sugars (mono and disaccharides) can be generally introduced into the cell as free sugars or as sugar phosphates. Disaccharides are then hydrolyzed to monosaccharides. Monosaccharides will subsequently enter one of the two main fermentative pathways: glycolysis or the phosphoketolase pathway [23].

Microbiological analysis of teff flour showed that about 90% of teff flour samples had aerobic mesophilic counts $\geq 10^5$ cfu/g and Gram-positive bacteria constituted about 71% of the total isolates. About 80% of samples had *Enterobacteriaceae* counts of 10^4 cfu/g [16]. Other study also showed that total microbial count of teff flour was $>10^5$ cfu/g, mainly consisting of yeasts (10^4 cfu/g), fermentative Gram-negative bacteria (10^4 cfu/g), aerobic spore formers (10^3 cfu/g) and lactic acid bacteria (10^3 cfu/g) [21].

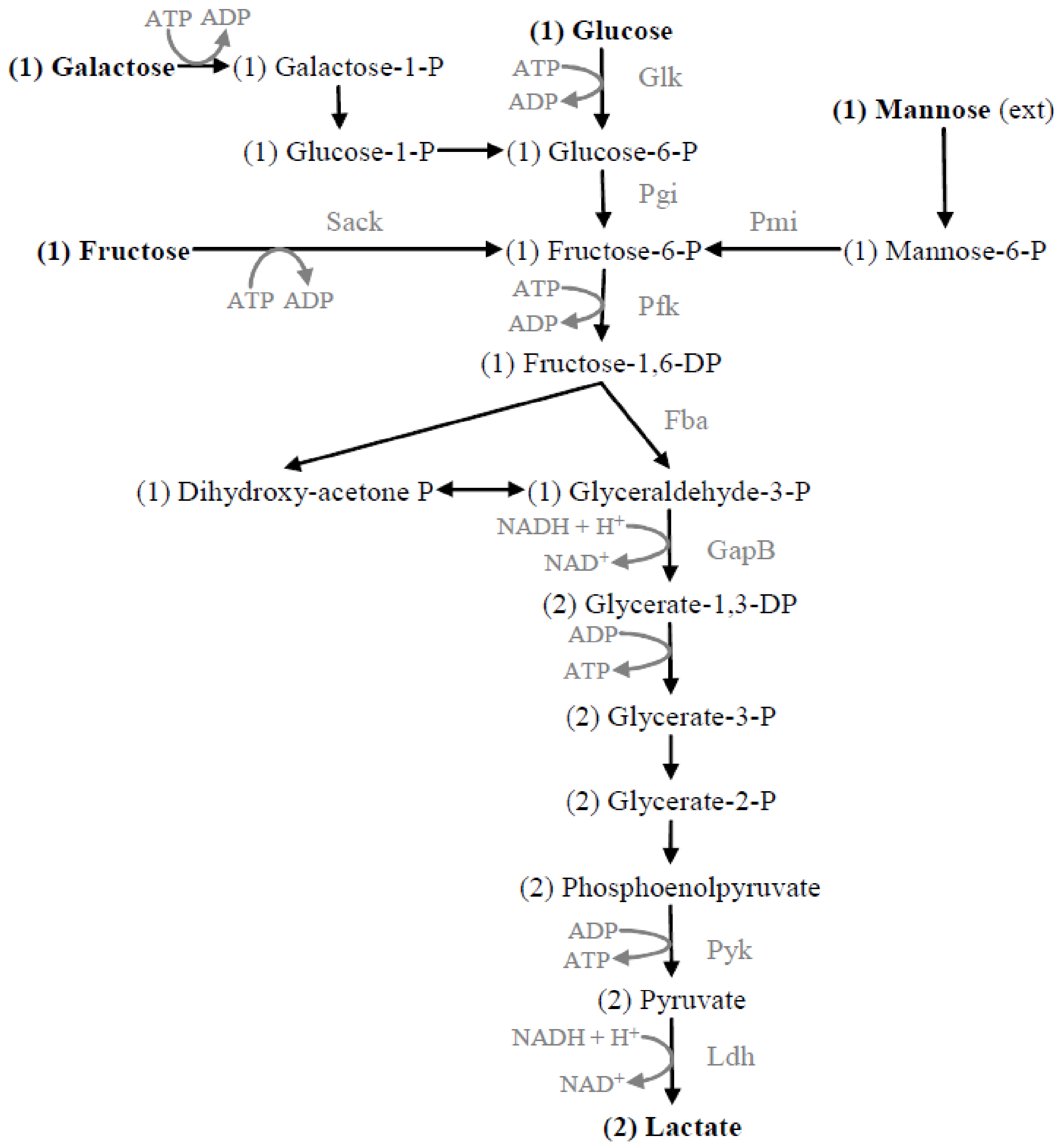

Figure 2: Glycolysis (Embden-Meyerhof-Parmas pathway). Abbreviations: Fba, fructose bisphosphate aldolase; GapB, glyceraldehydes-3-phosphate dehydrogenase; Glk, glucokinase; Ldh, lactate dehydrogenase; Pfk, 6-phosphofructokinase; Pgi, glucose-6-phosphate isomerase; Pmi, mannose-6-phosphate isomerase; Pyk, pyruvate kinase; Sack, fructokinase. Adapted from Carme 2007 [23]

One study on teff fermentation claimed that Gram-positive bacteria dominated the aerobic flora of teff and among these, *micrococci* and *Bacillus spp*. could be important in the initiation of the fermentation until members of *Enterobacleriaceae* could reach a large enough number to make any marked contribution to the fermentation, about 12 h [20]. The same study showed that members of *Enterobacteriaceae* were active during the first 18 hours of fermentation and reduced the pH of the fermenting dough to about 5.8. At this stage *Leuconostoc mesenteroides* and *Enterococcus faecalis* took over. Another study reported that as the pH of fermenting dough was further reduced to about 4.7, *Pediococcus cerevisieae*, *Lactobacillus brevis*, *Lactobacillus plantarum* and *Lactobacillus fermentum* became the dominant flora and remained so until fermentation was terminated at 72 hours [6]. The lactic acid bacteria were responsible for the acidic characteristics of the dough.

The yeast *Saccharomyces spp.*, even though present during the whole of the fermentation process, becomes abundant after the pH level reaches below 5 [22]. This yeast become the predominant group of organisms in the liquid layer after 50 hours of fermentation, (. after the complete separation of the liquid/solid) and is responsible for the rising of the dough during the second stage of fermentation [6, 21].

In a study of the yeast flora of fermenting teff, an average yeast count of $2x10^8$ cfu/g of dough after 22-24 hours of fermentation was observed [6]. The yeasts most prevalent in the yellow fluid belonged to the genera *Candida* and *Pichia*, and these were discarded with the yellow fluid. *Saccharomyces* and *Torulopsis* were the dominating flora during the secondary fermentation [24]. Other study showed that the pH of *ersho* samples was about 3.5 and titratable acidity ranged between 3.1% and 5.7%. Mean yeast counts ranged between $5.2x10^5$ and $1.8x10^6$ cfu/ml and comprised, in order of abundance, *Candida milleri*, *Rhodotorula mucilaginosa*, *Kluyveromyces marxianus*, *Pichia naganishii* and *Debaromyces hansenii* [16].

The very low pH values of the *ersho* (about 3.5) would be inhibitory for most kinds of micro organisms [6]. The absence of members of *Enterobacteriaceae* or lactic acid bacteria, which were reported to be important in teff fermentation [25], indicated that the *ersho* could not serve as a source of these bacteria [26].

Removal of the liquid layer (*ersho*) at the end of the primary fermentation removes soluble compounds [6]. A study showed that about 4-13% of teff nitrogen was lost depending on the stage of fermentation and suggested that this could be avoided by stopping the fermentation process before the liquid/solid separation [21].

All isolates of yeasts in *ersho* were known not to hydrolyze starch [6]. Thus they may not be active in the fermentation of teff until fermentable sugars are available due to the degradation of teff starch. They may, however, be important in leavening the dough of teff and producing flavour compounds in the latter stages of fermentation. No study has so far presented a conclusive proof as to which groups of microorganisms are important in breaking down starch and producing enough fermentable sugars to initiate the fermentation.

NUTRITIONAL AND HEALTH BENEFITS OF TEFF *ENJERRA*

Importance of Fermentation

Fermentation leads to synthesis and availability of nutrients, reduction in antinutritional factors and also leads to a general improvement in the shelf life, texture, taste and aroma of the final product [8, 11, 25, 27]. Lactic acid fermentation contributes towards the safety, nutritional value, shelf life and acceptability of a wide range of cereal-based foods [10].

Nutritional Importance of Teff Fermentation

Teff grain contains less than 1% (528-842mg/100g) phytic acid and other inositol phosphates, which are strong inhibitors of Fe and Zn absorption [28]. Fermentation gives *enjerra* its sensorial characteristics, as flavour, aroma and colour. But the more important effect of teff fermentation is the increase in the nutritional content, because of the decreasing relationships of iron with phytates and of iron and tannins [16]. One study on content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia reported that amount of phytates in *enjerra* is considerably reduced to 35-76 mg/100 g (91-93% destruction) due to fermentation and the acidity nature of *enjerra* [28]. Microorganisms are able to produce various metabolites during the fermentation through their enzymatic action on the substrate [18] and antinutritional factors are also reduced [6, 8, 16, 29]. Indeed, the lactic acid and volatile fatty acids (C2 - C6) were reported as the major organic acids produced during fermentation and contributing to good aroma and sour taste of *enjerra* [18].

When teff is used to make *engerra*, a short fermentation process allows the yeast to generate more vitamins specifically the B vitamins [6, 8]. The protein composition offers an excellent balance among the essential amino acids [29]. Different authors have demonstrated that during the teff paste fermentation, the phytate:iron molar ratio decreases. That is why, Ethiopian scientists and nutritionist agree about the need to improve the practice to ferment teff before use in *enjerra* production. Given the high iron content and the relatively favourable phytate:iron molar ratio, teff *enjerra* was the best source of bioavailable iron of all foods analysed by researchers, between the Ethiopian foods [16, 28].

A study on the carbohydrate composition of flour milled from red- and white-seeded teff varieties and the changes in carbohydrate content during fermentation showed that non-starch polysaccharides were largely unaffected by fermentation and baking [18]. Other study claimed fungal fermentation of teff improved the amino acid profile for the essential amino acids [4].

A study to investigate the effects of natural lactic acid fermentation of teff flour on HCl-extractability of minerals reported that natural fermentation of teff grain until the pH drops to about 3.84 and titratable acidity increases to 1.24 appears to be the most essential in reducing the phytate phosphorus concentration and phytate:zinc molar ratios as well as improving the extractability of iron, calcium, zinc and phosphorous [1]. Teff fermentation is therefore, an effective method of improving the HCl-extractability and possibly the bioavailability of minerals, which indicates nutritional importance of teff *enjerra*.

Teff *Enjerra* Health Benefits

Enjerra, particularly from red teff, has high iron content and in areas of the country where consumption of *enjerra* from red teff is prevalent, people tend to have higher levels of haemoglobin and, thus, a decreased risk of anaemia related to parasitic infection [16]. Since the teff grain is too small to separate into germ, bran and endosperm, the flour has much higher fibre content than other cereals. This is particularly important in dealing with diabetes in assisting with blood sugar control [6, 30, 31].

Interest in teff has increased noticeably due to its very attractive nutritional profile and gluten-free nature of the grain, making it a suitable substitute for wheat and other cereals in their food applications as well as foods for people with celiac disease [16, 31].

Research on celiac disease patients who are using teff reported a significant reduction in symptoms. This is possibly related to a reduction in gluten intake or to an increase in fibre intake [16]. Hence, teff *enjerra* can be a valuable addition to the gluten free diet of celiac disease patients.

The high level of iron in teff *enjerra* means that most people in Ethiopia eat their daily recommended nutrient intake of iron. But this does not mean that people in Ethiopia do not have anaemia, this is probably caused by a lack of foods that are rich in ascorbic acid, which improve the absorption of iron [32].

TEFF *ENJERRA* POTENTIAL AS FUNCTIONAL FOOD

Research has demonstrated that nutrition plays a crucial role in the prevention of chronic diseases. Functional foods are now known to contain bioactive substances, that play a role beyond the provision of energy and body forming, having the extra role of imparting health benefits to the consumer [33, 34, 35]. A food can be regarded as functional if it has beneficial effects on target functions in the body as a source of mental and physical well-being, contributing to the prevention and reduction of risk factors for several diseases or enhancing certain physiological functions, beyond adequate nutritional effects [34]. Functional components in functional foods include probiotics, prebiotics, soluble fibre, polyunsaturated fatty acids, antioxidants, vitamins, minerals among others [33].

In recent years, cereals and its ingredients are accepted as functional foods because of providing dietary fibre, proteins, energy, minerals, vitamins and antioxidants required for human health. Also, cereals can be used as fermentable substrates for the growth of probiotic microorganisms [34]. In many respects teff *enjerra* favours toward complete nutrient supply with functional food character for consumers, specially those with celiac disease [36].

Decrease in Antinutritional Factors

Fermentation of cereals could also decrease certain antinutritional factors like phytates, tannins, protease inhibitors and flatulence factors [4, 11, 37]. The antinutritional factors contribute to malnutrition and reduced growth rate due to the promotion of poor protein digestibility and by limiting mineral bioavailability [10, 38].

A study showed that antinutritional factors such as phytic acids, tannins and trypsin inhibitors decreased by 72%, 55% and 69%, respectively after teff fermentation for *enjerra* making [6]. Other study also claimed that the non-starch polysaccharides (dietary fibre) were not affected during teff fermentation [22].

The effect of fermentation on the bio-availability of iron, phosphorus and zinc of teff and wheat was studied and found out that fermentation increased the dialyzable portions of iron from 9% to 24%, phosphorus from 16% to 60% and zinc from 2% to 43% [16]. The researchers concluded that the increase in dialyzable iron might have a positive effect on its bioavailability, and might thus explain the rarity of iron-deficiency anaemia among teff consuming population of Ethiopia.

Antioxidant Activity

Antioxidants found in whole grain foods are polyphenols including phenolic acids and flavonoids, which are responsible for the high antioxidant activity [34, 39].

In a research carried out to evaluate the antioxidant content and activity of three teff varieties, it was observed that processing of teff flour into partly or fully fermented *enjerra* had reduced the total antioxidant contents with respect to raw teff flour but the total antioxidants retention capacity of partly fermented *enjerra* was higher than fully fermented *enjerra* [39]. From the study, it can be concluded that the *enjerra* fermentation process should be optimized in terms of antioxidant retention capacity as well as enhancing other functional factors.

Antimicrobial Activity

The production of organic acids, mainly lactic acid, reduces the pH, and as a consequence at the final stages of the fermentation, pathogenic bacteria are reported not to survive in the fermented food [6, 10]. Fermented foods do exhibit similar effect in the intestinal tract of the body, after consumption of the food [9].

The undissociated forms of the acetic and lactic acids at low pH exhibit inhibitory activities against a wide range of pathogens. This improves food safety by restricting the growth and survival, in fermented cereals, of spoilage organisms and some pathogenic organisms such as *Shigella, Salmonella* and *E. coli* [9, 11, 40, 41].

The preservative role of lactic fermentation in fermented products has been attributed to the production of acids, hydrogen peroxide and antibiotics [6]. Lactic acid production during fermentation of teff to produce *enjerra* has been found to lower the population of potential pathogenic organisms [42]. *Enjerra* also showed no aflatoxin contamination when prepared and handled. Fermentation of the dough or storage of *enjerra* for prolonged periods did not increase aflatoxin B1 contamination [43].

At normal *enjerra* fermentation (teff, 1% backslopping at 25°C) it took 12 h to reach a pH of 4 that could be regarded safe with respect to the prevention of the growth of pathogenic organisms [9]. The fermentation step in *enjerra* production, due to the prolonged (>48 h) processing at a pH <4 restricts the growth and survival of spoilage organisms and some pathogenic organisms such as *Shigella, Salmonella* and *E. coli* [6, 8, 9, 11, 40, 41].

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Probiotics

Probiotic bacteria such as *Bifidobacteria* and various *Lactobacilli* improve immune status of the colon, and are involved in preventing and depleting pathogenic and infectious bacteria in the gastrointestinal tract. Furthermore, probiotic bacteria are effective against lactose intolerance and diarrhoea [44]. These organisms are reported to have bacteriostatic, bactericidal, viricidal, anti-leukaemic and antitumor effects in the consumer [37, 45]. It is reported that fermented foods have a probiotic potential due to the probiotic *Lactobacillus* species that may be contained in them [46]. Probiotic microorganisms have great physiological impact as their fermentation of complex, non-digestible carbohydrates in the colon produces short chain fatty acids that have numerous benefits [44]. These short chain fatty acids are reported to play a role in prevention against colon cancer and other diseases [35, 44].

Lactobacillus plantarum is frequently associated with lactic acid fermented foods of plant origin, *eg* Ethiopian sourdough made from teff (*Eragrostis tef)* [47]. *Enjerra* from teff dough is one of the main lactic acid bacteria (LAB) fermented dietary sources in Ethiopia [48] and *Lactobacillus plantarum***,** *Lactobacillus brevis*, and *Lactobacillus fermentum* are regarded as the predominant species at the end of the fermentation of teff [20, 43], *Lactobacillus plantarum* being the most dominant [20, 43, 48]. Thus, individuals consuming *enjerra* also consume large amounts of *L. plantarum.*

A study on teff dough probiotic potential reported that lactic acid bacteria initiated the fermentation process at a level of 4 log cfu/g and reached about 9 log cfu/g at the end of fermentation period, and Lactic acid bacteria isolates showed that most of the isolates from the fermented product can survive the high acidity of the gastric environment and bile in the intestine, which are required properties of probiotic bacteria [49].

Improving teff fermentation processes for the production of probiotic and beneficial bacteria is a viable avenue for dealing with the challenges of diseases such as diarrhoea and the compromised status of gastrointestinal health in Ethiopia.

Prebiotics

A prebiotics is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota, that confer benefits upon host wellbeing and health [35]. Other suggested beneficial effects of prebiotics are improvement of calcium bioavailability and reduction in the risk for cardiovascular diseases, non-insulin dependant diabetes, obesity, osteoporosis, colon cancer as well as reduction of traveller’s diarrhoea [11, 13, 50].

Because of its small size, teff is made into whole-grain flour (bran and germ included), resulting in a very high fibre content and high nutrient content [16, 31], which can be taken as an indication to its prebiotic potential. If a prebiotic carbohydrate is utilised by a probiotic strain, its growth and proliferation in the gut will be selectively promoted [43]. So it could be argued that teff *enjerra* is potential synbiotic product, in that it contains LAB (potential probiotics) as well as dietary fibres (potential prebiotics).

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CONCLUSION

Teff fermentation for *enjerra* production is still conducted as spontaneous process (lacks appropriate starter culture). The art of traditional processes needs to be transformed into a technology to standardize quality of the end products without losing their desirable traits [51**]**. This may also pave the way for large-scale commercial production.

In light of its potential contribution to the improvement of general health and well being, the introduction of appropriate functional starter culture may constitute one major step towards improved safety, quality and functionality of teff *enjerra*. In a study conducted to explore the potential of using mixed culture fermentation to produce cereal-based foods with high numbers of probiotic bacteria, LAB growth was enhanced by the introduction of yeast and the production of lactic acid were increased in comparison against pure LAB culture [14]. A number of aspects of relevance for mixed starter culture development to enhance functional *enjerra* production should, therefore, receive special attention.

Dominant LAB and yeast strains could be further assayed in vitro and in vivo to ascertain their probiotic effects as guided by the 2002 FAO/WHO guidelines for evaluation of probiotics [52]. Such studies may involve but not be limited to: safety; evaluation of response to simulated stomach duodenum passage; susceptibility to clinically important antibiotics; hydrophobicity characteristics; adhesion to human cell linings; binding characteristics to human extra-cellular matrix; antinutritional factor reduction ability inclusive of other attributes claimed for probiotics.

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