Fermentation of teff (*Eragrostis tef*), grass-pea (*Lathyrus sativus*), and their mixtures: Aspects of nutrition and food safety.

Y. Yigzaw[1], L. Gorton[1], G. Akalu[2] and T. Solomon[3]

1. Department of Analytical Chemistry, Lund University, Lund, Sweden.
2. Ethiopian Nutrition and Health Research Institute, Addis Ababa, Ethiopia.
3. Department of Chemistry, Addis Ababa University, Addis Ababa, Ethiopia.

Email: (1) yirgalemy@hotmail.com

Introduction

Grass pea (*Lathyrus sativus*) is one of the important food legumes in countries like Bangladesh, India, and Ethiopia. It has desirable agronomic characteristics, notably the ability to resist adverse climatic conditions. Nutritionally it is tasty and protein-rich. However over consumption can cause an upper motor neuron disease known as neurolathyrism, an irreversible paraparesis of the lower limbs. The cereal grain teff (*Eragrostis tef* (Zucc.) Trotter) is one of the major cereal crops of Ethiopia, where it is believed to have originated. Teff provides over two-thirds of the human nutrition in Ethiopia [(3)], with a grain protein content (10-12%) similar to other cereals. Besides providing protein and calories, teff is a good source of minerals, particularly iron. It has a very high calcium content and contains high levels of phosphorus, copper, aluminium, barium and thiamine. The principal use of teff grain for human food is the Ethiopian bread "injera", a soft porous thin pancake with a sour taste.

The limited nutritional quality of cereal grains is due to their lower contents of proteins, fats, minerals, and vitamins compared with animal foods. It is an important fact that the amino acid composition of legume and grain seeds, both of which exhibit deficiencies of some of the essential amino acids for the human diet, are complementary. For example, cereal grains tend to be deficient in lysine whereas legume seeds are rich in this amino acid. The result is that blending of the two types of seed makes a nutritionally better food than does either alone. Fermentation of cereals and their blend with legumes is a potentially important processing method that can be expected to improve the nutritive value such as availability of proteins and amino acid profile. It could also decrease certain antinutritional factors like phytates, protease inhibitors and flatulence factors.

Summary of results

In the present study fermentation of pure teff, grass-pea and their mixtures, 9:1 and 8:2 (teff:grass pea) have been done at two temperatures (room and 35°C) in duplicate using strains of *Lactobacillus plantarum* for bacterial fermentation and both *Aspergillus oryzae* and *Rhizopus oligosporus* in succession for fungal fermentation. In addition two methods the natural or spontaneous and back-slopping (previously fermented culture used as a starter culture) methods of fermentation have been tried on the above four substrate groups. Although mixing teff with grass pea has not been part of the traditional practice for food preparation in Ethiopia, exploring the potential of fermentation of their blend may be beneficial. One obvious reason is developing an affordable nourishing crop for the poorer section of the population. However, in dealing with grass pea containing food products, the safety level has to be ascertained. This was the essence of the present work and we devoted much attention towards the development of analytical potential for an accurate evaluation of the toxin level after processing. In designing the experiment for fermentation we did not want to go higher than 8:2 (teff : grass pea) ratio as a compromise between nutritional adequacy and sensory value.

The pH and essential amino acid profiles of the different fermentation processes were compared. The toxin levels of both raw and fermented grass pea samples were also determined. The back-slopping with teff at a temperature of 35°C gave the sharpest pH drop. In all the cases, the fermentations done at elevated temperature showed a steeper slope in their pH vs. time plot compared to their room temperature counterparts. The spontaneous fermentation of pure *teff* and that using the pure culture of *Lactobacillus plantarum* at room temperature showed similar pH profiles, the former being slightly lower in pH at any time during the fermentation period. Both of them were slower fermentation processes as shown up in their pH profile (pH vs. time plot). The fermentation of pure grass pea with *Lactobacillus plantarum* at room temperature was the slowest among the substrate groups considered. One possible explanation for this could be the high protein content of grass pea, which renders a higher buffering capacity and hence slower change in pH. In a preliminary fungal fermentation experiment done, the trend in pH change was a progressive increase to more than 7. Such a trend is a common phenomenon in tempe fermentation, whereby tender-cooked soy bean or other legumes and legume/cereal mixtures are bound together in a white cake by mycelium of

the mould *Rhizopus spp.* It is already documented in literature that there is also a principle related to food safety in such high alkaline fermentations. A combination of high pH, free ammonia and rapid growth of the essential proteolytic microorganisms at relatively higher temperature, make it very difficult for other spoiling microorganisms to grow. In general a quick change in pH is a desirable phenomenon as it normally gives less chance for infecting microorganisms to compete with the desirable ones in a given setting.

The fungal fermentation improved the amino acid profile for the essential amino acids in all the substrate groups studied except for pure grass-pea substrate. Fermentation of teff:grass pea (8:2) , in particular has been found to be quite comparable in essential amino acid profile to an ideal reference protein recommended for children of 2 – 5 years old. It is important to note that the complementary effect of mixing cereals and legumes, which improves the inherent nutritional deficiencies of cereals and legumes alone, may have a contribution in the amino acid profile of the final product. On the other hand the bacterial fermentations (i.e. the spontaneous, back-slopping and those using *Lactobacillus plantarum* as inoculum) did not bring a net change in the essential amino acid profile.

The solid state fungal fermentation of pure grass pea was carried out with strains of *Rhizopus oligosporous* and *Aspergillus oryzae* in succession in that order at 35°C with autoclaving at the start and in between the inoculations at 100°C for 10 min [(1)]. The substrate was allowed to ferment for about 48 h with each inoculation (10^7 spores/5 g fermenting sample). Grass pea grits were subject to fermentation without any further sample pre-treatment, except adjusting the pH to 4 with lactic acid (50 $g.L^{-1}$). This fermentation process reduced β-ODAP level in grass pea on the average by 82% for the high toxin variety, and by up to 97% for the low toxin variety. β-ODAP levels were measured by a further optimised chromatography-biosensor coupled analysis method described below. The β-ODAP levels for the high and low toxin varieties of raw grass pea used for the present fermentation were originally 0.76 % and 0.52 % dry weight basis, respectively. The samples were collected in areas of Ethiopia where lathyrism is at a higher prevalence rate.

Analytical method

The chromatographic method of analysis employed refractive index in combination with bio-electrochemical detection for the simultaneous determination of the total amount of ODAP, selectively the amount of β-ODAP, and free glutamate. The biosensor construction for the electrochemical detection is based on crosslinking horseradish peroxidase (HRP) and an Os-containing mediating polymer as an inner layer and immobilizing L-glutamate oxidase (GlOx) as an outer layer on top of a graphite electrode. GlOx has a high activity primarily for L-glutamate but also for β-ODAP and aspartate. In the catalytic reaction the substrate is oxidized and the enzyme cofactor reduced. The natural reoxidizing agent is molecular oxygen, which in the reoxidation reaction of the enzyme is reduced to hydrogen peroxide.

To entrap the enzymes in the hydrogel and to prevent leakage of the mediator, a short chain polymer, poly ethylene glycol (400) diglycidyl ether (PEGDGE) was used as a cross-linking agent. Addition of polyethylenimine (PEI) in the hydrogel is believed to have sensitivity and stability enhancing effect on the biosensor. The double layer approach for the construction of the bienzyme sensor that avoids direct electrical wiring of GlOx has resulted in a high sensitivity of 4.6 $mA/M.cm^2$ and 14 $mA/M.cm^2$ for β-ODAP and L-glutamate, respectively. The system was quite linear over the range (1- 250 μM) for both glutamate and β-ODAP, the slope over this range was used to calculate sensitivities. Concentrations higher than these were not tried. The limits of detection for the chromatographic-biosensor system were found to be 2 and 0.7 μM for β-ODAP and L-glutamate, respectively. The refractive index detection on-line with the biosensor enabled full control of the chromatographic system for the determination of the total amount of ODAP, selectively the amount of β-ODAP and L-glutamate in raw and fermented samples containing grass pea. The non-β-ODAP selective spectrophotometric method of analysis developed by Rao [(2)] has also been employed on raw grass pea sample collections for the purpose of comparing results by the two entirely different methods of analysis. Because the Rao method uses alkaline hydrolysis of ODAP to DAP, it does not distinguish between the generally accepted non toxic α- and the toxic β-form. In literature it has been reported that the occurrence of α-form in raw grass pea does not exceed more than 5%. The Rao spectrophotometric method and the present method show an extraordinary degree of agreement as revealed by parallel “t” test (90 % confidence limit).

The present analytical system has operational stability of more than 50 h and storage stability of the biosensor about 4 days (dry, 4°C). Analysis time per sample is 10 min after extraction of ODAP which is completed within 90 min. (TRIS/KCl(10/10mM) buffer, pH 7). In general a fast, convenient and reliable analytical system is developed in the present study that could dependably measure the safety level of processed foods containing grass pea.

References

1. Kuo YH, Bau HM, Quemener B, Khan JK, Lambein F. (1995) Solid-state fermentation of *Lathyrus sativus* seeds using *Aspergillus oryzae* and *Rhizopus oligosporus* sp T-3 to eliminate the neurotoxin beta-ODAP without loss of nutritional value. J Sci Food Agric. **69**, 81-89.
2. Rao SLN. (1978). A sensitive and specific colorimetric method for the determination of alpha,beta-ODAP, the *Lathyrus sativus* neurotoxin. Anal. Biochem. **86**, 386-395.
3. Uraga K, Narasimha HV. (1997). Effect of natural fermentation on the HCl-extractability of minerals from Teff (*Eragrostis teff*). Bull. Chem. Soc. Ethiop. **11**, 3-10.