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The Effect of Fermentation on the Carbohydrates in Tef (*Eragrostis tef*)

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ABSTRACT

*Tef (*Eragrostis tef*) is a small seeded millet-like cereal grain indigenous to Ethiopia. It is commonly consumed as* injera, *a pancake like bread, made from fermented dough. The fermentation is generally spontaneous but may also be initiated by the addition of a starter culture from the previous fermentation.*

The carbohydrate composition of tef flour from both white and red seeded varieties and the changes that occur during domestic fermentation and baking have been investigated. Both varieties of tef contained ca. *2·7 g/100 g DM of free sugars, predominantly sucrose (95%). Fermentation initially increased the amounts of free sugars; thereafter the total fell. The changing pattern of free sugars during fermentation was the same in both varieties and was due to changes in the microbial population dynamics resulting from changes in dough pH. Fructose was found to be the principal free sugar in the fermenting dough and cooked product. After 72 h fermentation, the microbial population had utilised 9% of the starch in both varieties. The non-starch polysaccharides (NSP) (dietary fibre) were unaffected.*

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INTRODUCTION

Tef (*Eragrostis tef*) is a small millet-like cereal grain indigenous to Ethiopia where it is extensively cultivated for human food. It is occasionally grown in other countries, e.g. South Africa and Australia, as a forage crop.

In Ethiopia, tef is the single most important cereal crop and is used to make a variety of commonly consumed food items, i.e. *injera* (a pancake-like bread), porridge, *tella* (local beer) and *katikalla* (local spirit). Although *injera*, a fermented and baked product, can be made from other cereal flours or flour mixtures, that made from tef is preferred, the consumption being up to three times greater than that made from other cereals (Ethiopian Nutrition Survey 1959).

In the process of making *injera* the dough is subjected to a two-stage fermentation. The first fermentation, which may last 24 to 72 h, starts spontaneously when the flour is wetted due to the high level of contaminating organisms or it may be initiated by the addition of *irsho* (a thin paste saved from the previous fermentation). The microorganisms are partly the natural flora of the cereal grain and partly contamination by Enterobacteria from animal dung used to prepare the threshing floor. The time for optimum fermentation, i.e. when gas production ceases and the dough and liquid phase separate, varies, depending on how the fermentation is initiated, the numbers and type of organisms present in the *irsho* or flour, ambient temperature and the type and bacterial cleanliness of the container used (Stewart & Getachew, 1962). At the end of the first stage of fermentation the surface liquid layer is discarded.

A portion (about 1/10) of the dough is removed, thinned to a paste and boiled for a few minutes to make *absit*. The hot *absit* is added back to the bulk of the dough and mixed in to initiate the secondary fermentation which usually lasts about one hour. The fermented dough, which has the consistency of batter, is poured onto the hot oiled surface of the *metad*, which is a round smooth clay griddle, and baked for a few minutes to produce the large pancake-like *injera*.

Although some information is available on the microorganisms responsible for the fermentation of tef (Gashe *et al.*, 1982; Gifawosen & Besrat, 1982; Gashe, 1985) there is no information on the nutritional changes brought about by fermentation. The purpose of this study was to obtain data on the changes in carbohydrates that occur during the preparation of *injera*, fermented by the organisms normally present in the flour.

MATERIALS AND METHODS

Two varieties of tef seed, white and red, were obtained through the Ethiopian Nutrition Institute, Addis Ababa. The samples were ground to a

coarse flour in an ultracentrifugal mill to pass a 0·5 mm screen. Duplicate fermentations were carried out by mixing 2:3 w/w flour:water (distilled, sterile) and incubating at 22°C. Duplicate samples were taken from each fermentation at 0, 24, 48 and 72 h (after discarding the supernatant liquid phase), at the end of the secondary fermentation and after baking. One set of samples were freeze-dried, ground to a homogeneous powder in a mortar and stored in air-tight bottles at room temperature until analysed. The remaining set of samples were used for microbial counts and pH measurements.

Free sugars were extracted from 5 g of the dry sample using 3 × 25 ml boiling 80% ethanol and made to 100 ml. The alcohol-insoluble residue (AIR) was further extracted 2 × 25 ml boiling acetone to remove residual water, fat and pigment. The dry AIR was weighed, ground in a mortar and stored in air-tight bottles prior to analysis.

Individual free sugars were separated and quantitatively determined by HPLC (Zygmunt, 1982). The starch and non-starch polysaccharides were estimated using a modified procedure of Englyst (Englyst *et al.*, 1982). The enzymically hydrolysed starch fraction was retained and the starch estimated as glucose equivalents by the method of Roe (1955).

Yeasts, lactic acid bacteria and total aerobic organisms were enumerated by inoculating 0·1 ml of serial dilutions (10^{-1}–10^{-8}) of the samples in sterile water onto oxytetracycline–dextrose yeast (ODY), Rogosa and Cysteine–Lactose–Electrolyte Deficient (CLED) plates, respectively. ODY plates were incubated at 25°C, CLED at 30°C and Rogosa at 25°C in an anaerobic jar. Counts were made after 72 h incubation.

RESULTS AND DISCUSSION

Microbiology

Both varieties of tef were similar in the broad categories of organisms enumerated. The population dynamics are shown in Fig. 1. The predominant organisms present in the flour were yeasts; however, the numbers of both lactobacilli and aerobic organisms increased rapidly during fermentation with both groups of organisms starting to decline at the end of fermentation. Yeasts, however, increased only slowly up to 48 h, during which time the pH fell from 6·3 (0 h) to 4·3 (48 h) and thereafter increased rapidly up to the end of the secondary fermentation where the pH was 3·9.

Free sugars

The free sugar content of the tef flours and doughs during fermentation and after baking are given in Table 1. The predominant sugar in the raw flour is

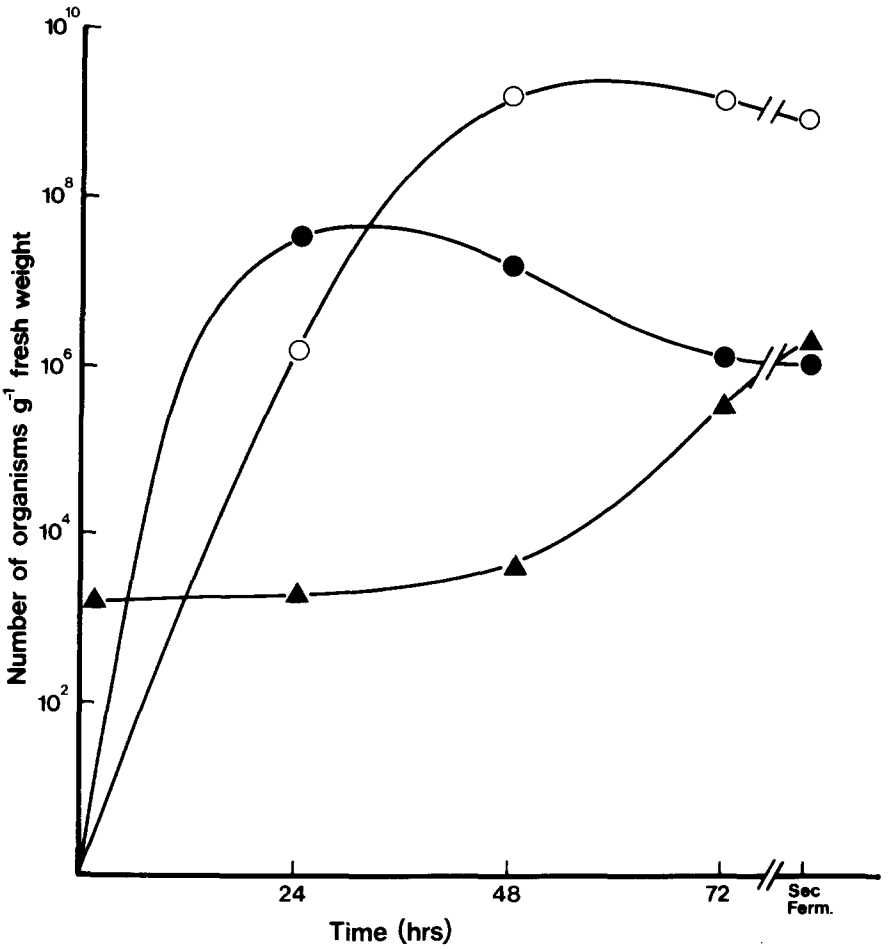

Fig. 1. Changes in the numbers of broad groups of organisms during fermentation of tef. ●—●, Aerobic organisms; ○—○, lactobacilli; ▲—▲, yeasts.

sucrose (95%) with a small amount of maltotriose. Immediately after mixing the flour and water there is rapid production of fructose, maltose and maltotriose. Both maltose and maltotriose are probably derived from starch by the action of endogenous amylases present in the flour. The fructose may be derived from fructosans or synthesised by some other mechanism, e.g. isomerisation of glucose, but not sucrose as this remains almost unchanged.

After 24 h the free sugars reach a maximum, the predominant sugar being fructose (*ca.* 60%), some of which may well be derived from sucrose since the amount of this sugar fell sharply. Maltose and maltotriose also peak at 24 h. Between 24 and 72 h all the free sugar levels fell, probably as a result of utilisation by the increasing microbial population (Fig. 1). Glucose was only

TABLE 1
Free Sugar Composition during Fermentation and after Baking (g/100 g DM) (Percentage of Total Sugars in Parentheses)

Fermentation period (h)	*Fructose*	*Sucrose*	*Maltose*	*Maltotriose*	*Total*
White tef					
Flour	0	1·3 (93)	0	0·1 (7)	1·4
0	0·4 (16)	1·3 (52)	0·5 (20)	0·3 (12)	2·5
24	3·1 (62)	0·2 (4)	0·7 (14)	1·0 (20)	5·0
48	2·2 (85)	0	0·2 (8)	0·2 (8)	2·6
72	1·6 (80)	0	0·2 (10)	0·2 (10)	2·0
SF	3·4 (87)	0	0·5 (13)	0	3·9
baked	3·7 (93)	0	0·3 (7)	0	4·0
Red tef					
Flour	0	1·8 (95)	0	0·1 (5)	1·9
0	0·3 (11)	1·7 (63)	0·3 (11)	0·4 (15)	2·7
24	1·3 (62)	0·1 (5)	0·7 (33)	0 ·	2·1
48	0·8 (62)	0	0·4 (31)	0·1 (8)	1·3
72	0·6 (60)	0	0·2 (20)	0·2 (20)	1·0
SF	2·7	0	0	0	2·7
baked	3·2	0	0	0	3·2

found in trace amounts at all time points and this would indicate either very rapid utilisation or lack of synthesis. In the anaerobic fermentation of carbohydrate foods, e.g. *bouza*, as carbohydrate is utilised there is production of lactic and volatile fatty acids, ethanol, nitrogen, carbon dioxide and hydrogen (Morcos *et al.*, 1973). This would appear to be similar to the situation observed in tef where the lactic acid bacteria caused the pH to fall from 6·3 to 3·9 accompanied by gas production and the characteristic odour of volatile fatty acids.

By the end of the secondary fermentation free sugars had increased to levels similar to those found at 24 h despite the increased microbial numbers (Fig. 1). This probably results from acid hydrolysis of the more labile polysaccharides in the *absit*, the pH of which was *ca.* 4·0. Further, dilution of the dough will reduce the concentration of growth-inhibiting substances produced by the microbial population and gelled starch will be more readily hydrolysed by the amylases in the dough.

The small changes in free sugar content of the *injera* are a result of thermal degradation of polysaccharides on cooking.

It is of interest to note that both maltose and maltotriose in the red tef were lower than observed in the white variety. It is known that many cereals,

particularly those with red–brown bran layers, may contain high levels of tannin, a known inhibitor of α-amylase (Tamir & Alumot, 1969; Strumeyer & Malin, 1969) and this may have been the cause since light microscopy showed that red tef contained large amounts of tannins in the bran layer.

Starch

The changes in starch content are given in Table 2. It would appear from this data that starch was utilised as the main energy source by the fermentation organisms, resulting in a 9% loss of starch in both types of tef. This is similar

TABLE 2
Changes in Starch Content during Fermentation and after Baking in White and Red Tef (g/100 g dry matter)

Fermentation period (h)	*White tef*	*Red tef*
0	78·5	78·7
24	74·8	74·9
48	73·8	73·9
72	71·9	72·1
secondary ferm.	70·6	71·5
baked	69·6	69·6

to the fermentation of cow peas (Zomara & Fields, 1979) and sorghum (El Tinnay *et al.*, 1979; Kazanas & Fields, 1981). The abrupt increase in the rate of loss of starch between the 72 h and secondary fermentation probably results from the rapid fermentation of the gelled starch present in the *absit.* It is unlikely that the loss of starch is nutritionally significant since much of the carbohydrate appears to be incorporated into bacterial mass or to be present as lactic and volatile fatty acids, all of which will provide energy when ingested.

Non-starch polysaccharides (NSP)

The monomeric sugar and uronide composition are given in Table 3. Both varieties of tef flour were found to be low in NSP (*ca.* 5%) by comparison with wheat and to contain a relatively high proportion of glucuronic acid, which is similar to rice (R. M. Faulks, pers. comm.) but unlike other major

TABLE 3
Changes in Dietary Fibre Components during Fermentation and after Baking of Tef (g/100 g DM)

Components	*0 h*	*24 h*	*48 h*	*72 h*	*SF*	*Baked*
White tef						
Non-cellulosic Polysaccharide						
Arabinose	0·9	0·9	0·7	0·8	0·8	0·8
Xylose	0·9	0·8	0·7	0·7	0·7	0·6
Mannose	0·1	0·1	0·1	0·1	0·1	0·1
Galactose	0·3	0·3	0·3	0·3	0·3	0·3
Glucose	1·1	1·1	1·2	1·1	1·2	1·1
Uronic acid	1·2	1·2	1·3	1·3	1·2	1·3
Total NCP	4·5	4·4	4·3	4·3	4·3	4·2
Cellulose	1·0	1·1	1·0	1·1	1·1	1·2
Lignin	—	—	—	—	—	—
Total dietary fibre	5·5	5·5	5·3	5·4	5·4	5·4
Resistant starch	0	0·1	0·1	0·1	0	0·1
Red tef						
NCP						
Arabinose	0·7	0·7	0·8	0·7	0·7	0·7
Xylose	0·6	0·5	0·6	0·6	0·6	0·6
Mannose	0·1	0·1	0·1	0·1	0·1	0·1
Galactose	0·3	0·3	0·3	0·3	0·3	0·3
Glucose	1·1	1·1	1·1	1·2	1·2	1·1
Uronic acid	1·2	1·2	1·2	1·2	1·3	1·2
Total NCP	4·0	3·9	4·1	4·1	4·2	4·0
Cellulose	1·2	1·1	1·2	1·1	1·1	1·0
Lignin	—	—	—	—	—	—
Total dietary fibre	5·2	5·0	5·3	5·2	5·3	5·0
Resistant starch	0·1	0·1	0·1	0	0	0·2

cereals. The relatively high proportion of glucose in the non-cellulosic fraction would indicate the presence of some β-glucans as in oats and barley.

No significant differences were found either in total amount or composition of NSP during fermentation and cooking, indicating that the cell wall polysaccharides are not utilised by the fermentation organisms.

Starch, resistant to enzymic hydrolysis in the destarching stage using α-amylase and pullulanase, was estimated in the NSP fraction and found to be negligible.

CONCLUSION

During the fermentation of tef flour to make *injera*, starch is the major source of energy utilised by the fermenting organisms. The total and relative amounts of free sugars found during fermentation are a result of changes in the production and activity of endogenous and extracellular bacterial enzymes and the utilisation of the sugars by the changing microbial population. The non-starch polysaccharides are not utilised.

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REFERENCES

El Tinnay, A. H., Abdul Gadir, A. M. & El Hadia, M. (1979). Sorghum fermented Kisra bread. 1. Nutritive value of Kisra. *J. Sci. Fd Agric.*, **30**, 859–63.

Englyst, H., Wiggins, H. S. & Cummings, J. H. (1982). Determination of non-starchy polysaccharides in plant foods by GLC of constituent sugars as alditol acetate, *Analyst*, **107**, 307–18.

Ethiopian Nutrition Survey (1959). A report by the Interdepartmental Committee of Nutrition on National Defence.

Gashe, B. A. (1985). Involvement of lactic acid bacteria in the fermentation of tef (*Eragrostis tef*), an Ethiopian fermented food. *J. Fd Sci.*, **50**, 800–1.

Gashe, B. A., Girma, M. & Besrat, A. (1982). Tef fermentation. 1. The role of micro-organisms in fermentation and their effects on the nitrogen content of tef, *Sinet. Ethiop. J. Sci.*, **5**(2), 67–76.

Gifawosen, C. & Besrat, A. (1982). Yeast flora of fermenting tef (*Eragrostis tef*) dough, *Sinet. Ethiop. J. Sci.*, **5**(1), 21–5.

Kazanas, N. & Fields, M. L. (1981). Nutritional improvement of sorghum by fermentation. *J. Food Sci.*, **46**, 819–21.

Morcos, S. R., Hegazi, S. M. & El-Damhoughy, S. T. (1973). Fermented foods of common use in Egypt. II. The chemical composition of bouza and its ingredients. *J. Sci. Fd Agric.*, **24**, 1157–61.

Roe, J. H. (1955). The determination of sugars in blood and spinal fluid with anthrone reagent. *J. Biol. Chem.*, **212**, 335–43.

Stewart, R. B. & Getachew, A. (1962). Investigation of the nature of injera, *Econ. Bot.*, **16**, 127–30.

Strumeyer, D. H. & Malin, M. I. (1969). Identification of the amylase inhibitor from seeds of Leoti sorghum. *Bioch. Biophys. Acta*, **184**, 643–8.

Tamir, M. & Alumot, E. (1969). Inhibition of digestive enzymes by condensed tannins from green and ripe carobs. *J. Sci. Fd Agric.*, **20**, 199–203.

Zomara, A. F. & Fields, M. L. (1979). Nutrition quality of fermented cowpeas and chick peas. *J. Fd Sci.*, **44**, 234–6.

Zygmunt, L. C. (1982). HPLC determination of mono and disaccharides in presweetened cereals. *J. Assoc. Off. Anal. Chem.*, **65**(2), 256–64.