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Research Paper

Production of organic acids, titratable acidity and pH-development during fermentation of cereal flours

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ABSTRACT

Following the household method of fermentation used in making the Ethiopian bread injera, fermentation of a flour/water slurry was carried out. The fermentation experiments were done at temperatures of 25 and 35 °C with whole grain wheat flour, whole grain tef (*Eragrostis tef*) flour and commercial bakery flour of 70% extraction rate. The slurry was made by mixing 300 g flour and 600 ml water. Backslopping was done by adding a portion of the slurry which was fermented in a previous batch as a starter (0.1%, 1.0%, and 10%) to the next batch to be fermented. During the fermentation, samples were taken at definite intervals of 3, 6, 9, 12, 18, 24, 48 and 84 hours respectively and kept frozen until they were used for analysis. The pH and titratable acidity were recorded and the organic acids were determined by HPLC using an Aminex® HPX-87H column, The growth of lactic acid bacteria including the typing of species/strains was also determined. Samples of the fermenting slurry were taken for analysis and the final concentration of lactic acid was found to be approximately 1 g per 100 g. A higher amount of inoculum gave a quicker lowering of pH, and also a lower initial pH. A smaller inoculum amount showed a delayed start in the lowering of pH. A higher temperature increased the production rate of lactic acid.

The dominant (100% at 25°C) species of the final sample of fermented tef slurry was found to be ***Lactobacillus plantarum*.**

Commercial bakery flour showed a comparatively low buffering capacity, less than 0,1 g lactic acid/100 g was needed to reduce pH to <5. Tef, a cereal with very small seeds contributing a larger portion of outer parts of the seeds in the flour, had the highest buffering capacity. In the region pH 6 to 4, approximately double the amount of lactic acid was required in comparison to the commercial bakery flour in order to attain the same pH. This indicates that at the same pH-level, a fermented food item made of tef, and also of whole grain wheat, probably has a higher food safety than a food item made of commercial bakery flour. Thus, the use of whole grain flour improves not only the nutritional quality, but also the food safety of a fermented food, which is of importance under household conditions of low-income countries.

At normal injera fermentation (tef, 1% backslopping at 25°C) it took 12 h to

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reach a pH of 4 that could be regarded safe with respect to the prevention of the growth of pathogenic organisms.
The fermentation step in injera production, due to the prolonged (>48 h) processing at a pH <4, should give the product a high food safety regarding pathogenic microorganisms usually encountered in foods.

Keyword: Lactic acid, Farmentation, *Lactobacillus plantarum*

Introduction

Fermentation is used all over the world for processing and preservation of various foods. During the last few years, fermentation of foods has been a focus of discussion due not only to its probiotic effects but also to the food safety aspects. Diarrhoeal diseases are an important cause of infant mortality along with respiratory infections (Motarjemi *et al.*, 1993). In many countries of Africa, fermented gruel based on various cereal flours is used for feeding infants. Water is a source of contamination mainly in the poor income societies. Lactic acid fermentation, when it is properly done, could be used for preventing the growth of enteropathogenic bacteria to a significant level. 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 (FDA, 1992). Fermented foods do exhibit similar effect in the intestinal tract of the body, after consumption of the food. Lactic acid fermented gruel fed to young children has been shown to significantly reduce the number of enteropathogenic bacteria in faecal swabs of the children (Kingamkono *et al.*, 1999). A comparison of the prevalence of diarrhoea among the children of two villages with different feeding patterns, showed that the children of the village which used lactic acid fermented gruel had a lower prevalence of diarrhoea among them compared to the children of the village which used non-fermented gruel, (Lorri & Svanberg, 1994). In addition to the effect of lactic acid and pH on the growth of enteropathogenic organisms, many other positive effects of fermentation have also been found by various authors. Fermented foods containing lactic acid was reported to give low glycemic index compared to non fermented foods (Liljeberg *et al.*, 1995). Low glycemic index is favourable for better feeling of satiety and low insulin requirement, not only for non insulin dependent diabetics but also for normal healthy individuals. The overall nutritional value of raw materials could be improved by production of certain vitamins (Shahani, 1983) and essential amino acids (Hesseltine & Wang, 1980) during fermentation. Reduction of the antinutritional substances like phytic acid and polyphenols in cereals and legumes (Khetarpaul & Chauhan, 1991), production of immunosupressive compounds and antibiotics like nicin used as preservatives (Gould, 1995) are also found in connection with food fermentation. Reduction and in some cases complete inactivation of cancerogenic substances like aflatoxin (Ogunsanwo *et al.*, 1989), and other toxic substances like ß-ODAP (Yu Haey Kuo *et al.*, 1995), by various microorganisms have also been reported.

Fermentation is also an environment friendly, energy effective process of food preservation which can be used in a sustainable manner especially in low income societies. Therefore a thorough knowledge of the different factors governing the process in relation to food safety, nutritive value and sensory properties are extremely valuable in optimisation of the process in household level as well as in an industrial scale.

The processing conditions applied in fermenting food under household conditions varies from one part of the world to the other to a great extend regarding the water to solids ratio, type of microbial flora present or added, the type of raw material used and the type of finished product expected. Very often a spontaneous fermentation utilising the microflora present on the raw materails is used for initiating fermentation. Sometimes a kind of starter, which is a previously fermented product, is used not only to initiate the fermentation but also to keep a uniform quality from one day to another. In addition to cereals, fish, meat, dairy products, fruits and vegetables are also fermented either alone or in combinations. Sometimes fermented foods are given some characteristic sensory properties by addition of spices, condiments and salts.

The present study deals with the changes that are taking place during fermentation of slurries made of cereal flour and water. This is a common method for producing fermented food not only for adults but also for infants in many African countries. A common Ethiopian food item called “injera”, a circular, flat, spongy, pancake like, bread that is made of fermented slurry of the flour of the cereal tef (Egragrostis tef) was chosen as a model for our investigation. Tef is a major cereal in Ethiopia having very small grains, cultivated on more than 50% of Ethiopia’s total cereal area in 1980 (Lost crops of Africa, 1996). A recipe taken from an Amharic cookbook was scaled 1/10 to make a laboratory scale. Injera is produced by mixing approximately one part of flour with two parts of water and adding a starter taken from the previous fermentation (backslopping). This gives a rather thick slurry, which after a few hours settling gets a thin protective top layer of transparent liquid. The slurry is left to ferment for two to three days. The fermented slurry is poured on a hot pan to a thin layer and heated from below till it is ready for eating. Before cooking a small portion of the slurry is mixed with water and cooked to a paste and added back and mixed well to give the slurry the necessary consistency. The fermented slurry is also cooked to a gruel for use as a breakfast food item.

The objectives of the present experiment was to follow the changes in the production of organic acids, development of pH, amount of titratable acidity. Fermentation was carried out with bakery wheat flour, whole grain wheat flour and tef flour, at temperature 25°C and 35°C and different amounts of starter for initiating the fermentation process.

Materials and methods

Materials

Commercial quality wheat flour of 70% extraction rate designated as "bagerivetemjöl" was obtained from the industrial flour mill Skånemöllan AB, Sweden. Whole grain wheat of the variety Kosack was kindly provided by the seed company Svalöv/Weibulls AB, Svalöv, Sweden. Tef grains (*Eragrostis tef*) of the DZ-01-196 variety was bought from the Bio-Diversity Institute of the Federal Government of Ethiopia, Addis Ababa, Ethiopia.

Lactate standard 40 mg/dl (ref no. 826-10) was obtained from Sigma. Organic acid analysis standard with 0,8 µmol sodium oxalate, 4,0 µmol sodium citrate, 8,0 µmol sodium malate, 20,0 µmol sodium succinate, 20 µmol sodium formate and 20,0 µmol sodium acetate, was obtained from Bio-Rad (no. 125-0586). All other chemicals used were of analytical grade.

Methods

Sample preparation for fermentation

To obtain the flour from tef and whole wheat, the grains were cleaned by rinsing three times in distilled water, dried in a ventilated oven at 50°C and milled in a Tecator Cyclotec mill using a 1-mm sieve. The flour was stored in a closed plastic container at 4°C until it was used for further experiments.

Fermentation

Fermentation was performed in a slurry made with 300 g of flour mixed with 600 ml of 40°C warm water in a 1-l beaker. To get a homogenous mixture, 250 ml of water was first added to the flour and mixed, then two subsequent portions of 75 ml water was added with mixing in between. Finally, the remaining 200 ml was added together with the inoculum and thoroughly mixed. The beaker was placed in a water bath with a constant temperature of 25°C or 35°C and the fermentation was carried out without stirring, in accordance with the usual household practise.

Three different amounts were used, 1 g (»0,1%), 10 g (»1%) and 100 g (»10%) for backslopping. The fermentation was started without inoculum as a spontaneous fermentation, and then backslopping was performed at 84-h intervals. Three consecutive backsloppings were made before the microbial flora was adapted and the system was considered to be consistent (Nout *et al.*, 1989) to allow samples being taken for analysis. The inoculum was taken from the previous batch after the liquid top layer above the sediment had been decanted.

Sampling

Samples were withdrawn at the beginning of fermentation and after every 3, 6, 9, 12, 18, 24, 48 and 84 hours respectively. Using a syringe-like sampling device, 10 ml of the sample was taken at level 1 cm above the bottom surface of the beaker. The samples were quickly frozen by placing each of them in a plastic container

directly on a refrigerated shelf of a freezer. The frozen samples were kept at -18°C till they were used for further analysis.

For testing which type of lactic acid bacteria was present in the tef fermentation, samples were taken at the end of the fermentation process. These samples were kept refrigerated at 4°C till they were used for further analysis.

Sample preparation for analysis

The frozen sample was thawed to room temperature, and approximately 1 to 2 g in duplicates were weighed into a centrifuge tube to which 200 µl of formic acid (12,1 mg/ml) was added as internal standard together with 7 ml of distilled water. The content was homogenised and placed in a water bath of 65°C for 5 minutes to prevent the lactic acid bacteria from continuing the fermentation process during the analyses.

The centrifuge tube with its contents was cooled to room temperature in an ice bath, neutralised to pH 7 with 0,1 M sodium hydroxide, and water was added to adjust the volume to 10 ml. The tube was then placed in an ultrasonic bath for 5 min to facilitate the extraction of the organic acids, and then centrifuged at 4000 rpm. (Johansson *et al.*, 1995) The supernatant in the centrifuge tube was filtered through a 45-µm filter (Millipore HAWP 02500) and 20 µl of the filtrate was injected in the HPLC column.

Measurement of pH

The pH was measured using an Orion expandable ion analyzer EA 920 and an Orion Sure-Flow Ross pH-electrode. The electrode was carefully immersed into and removed from the beaker, not to disturb the fermentation more than necessary. Measurements of pH were made at the same time intervals as the sampling.

Analysis of organic acids

Organic acids were analysed using an HPLC-apparatus consisting of a Pharmacia Pump P-3500, an Aminex® HPX-87H column from Bio-Rad Laboratories and a Pharmacia Liquid Chromatography Controller LCC 500. As the mobile phase 0,005 M sulphuric acid was used at a flow rate of 0,6 ml/min. The column was kept immersed in a water bath kept at 35°C. For detection a Varian 2550 UV-detector at 410 nm set at range 0,16 was used. Recordings were made on a Pharmacia Two-channel Recorder REC-482 and on the LCC 500. Formic acid was used as internal standard and it did not interfere with the separation of other acids. Standard curves for lactic acid were plotted using peak height and peak area given by the LCC 500, and peak height was chosen to be used as it showed the best correlation. All analysis was done in duplicates.

Titratable acidity

The titratable acidity was measured by titrating a mixture of 3 g of sample and

27 ml of distilled water to pH 8.5 using 0.1 M sodium hydroxide solution (Kingamkono *et al.*, 1994). The result was expressed as g lactic acid/100 g sample, and plotted against the results from HPLC-analysis. The trendline for the plot was established using Microsoft Excel.

Microbiological assay for typing the flora in the fermented slurry

For the microbiological assay, samples were taken from fermented slurry of tef after 84 hours of fermentation. The samples were diluted ten times by volume with isotonic saline solution.

To determine the number of lactic acid bacteria the samples were plated on Rogosa agar (Difco, Detroit, Michigan, USA) and incubated at 37°C for 48 h under anaerobic conditions (BBL Gas Pak Anaerobic System, Becton Dickinson Microbiology Systems, Cockeysville, Maryland, USA). Colonies were randomly picked, purified and stored in freezing buffer (Ahrné *et al.*, 1989). From each of these samples five isolates were drawn for identification and subtyping.

Identification and subtyping of *Lactobacillus plantarum* was done by Randomly Amplied Polymorphic DNA (RAPD) according to the method described by Johansson et al, (Johansson *et al.*, 1995) and by API 50 CH (API System, Montalieu, Vercieu, France).

Results

Organic acids

Figure 1a shows the chromatogram with separation of the standard organic acids which may be found in fermenting organic material. In Figure 1b the chromatogram of standard lactic acid with internal standard formic acid is shown. The response for lactic acid was linear between the values corresponding to 0,06 and 0,96 g/100 g with an R^2 value of 0,9999. Formic acid used as internal standard separated well from the other acids and under normal circumstances it is not likely to be present in fermenting cereals. Figure 1c-f shows the separation of organic acids from nonfermented samples and fully fermented samples of commercial bakery flour (bagerivetemjöl) and tef. The detection level at the used instrumental setup was found to be equivalent to 0.01 g lactic acid/100 g sample and 0,005 g acetic acid/100 g sample. Only the amount of lactic acid was quantified and presented since no peaks that could be attributed to other acids were found.

Final concentration of lactic acid

The total amount of lactic acid at the end of the fermentation was for tef slurry fermented at 25°C approximately 1,0 g per 100 g (Figure 2). At the higher temperature, 35°C, the amount was slightly lower. The whole grain wheat flour slurry had a different pattern, where at 25°C the final amount was 0,6–0,8 g per 100 g while at 35°C it was 1,0–1,2 g per 100 g (Figure 3). For bagerivetemjöl the

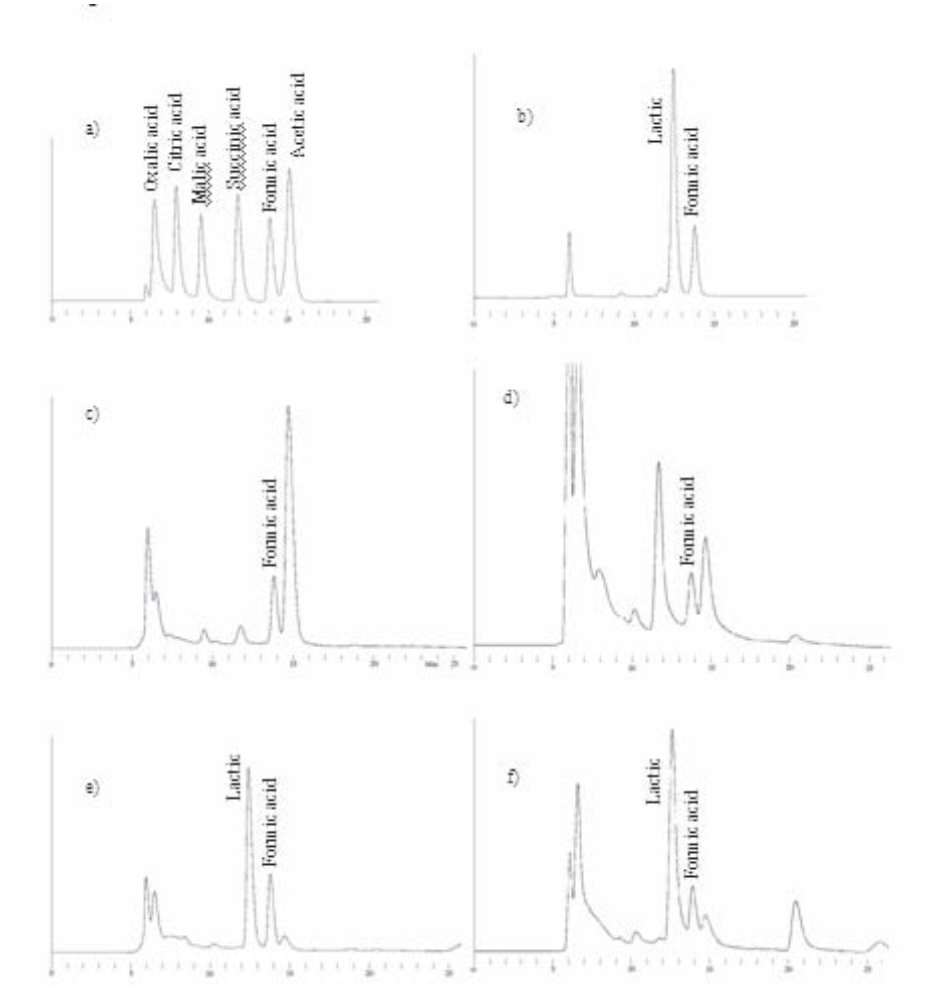

Figure 1: HPLC-charts: a) standard acids, b) lactic acid standard, c) nonfermented bagerivetemjöl, d) nonfermented tef, e) fermented bagerivetemjöl, f) fermented tef

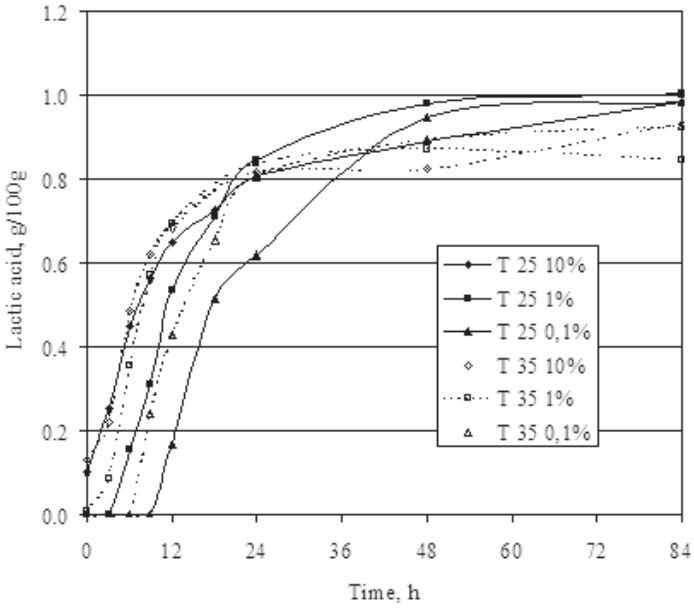

Figure 2. Lactic acid content in fermenting tef slurry

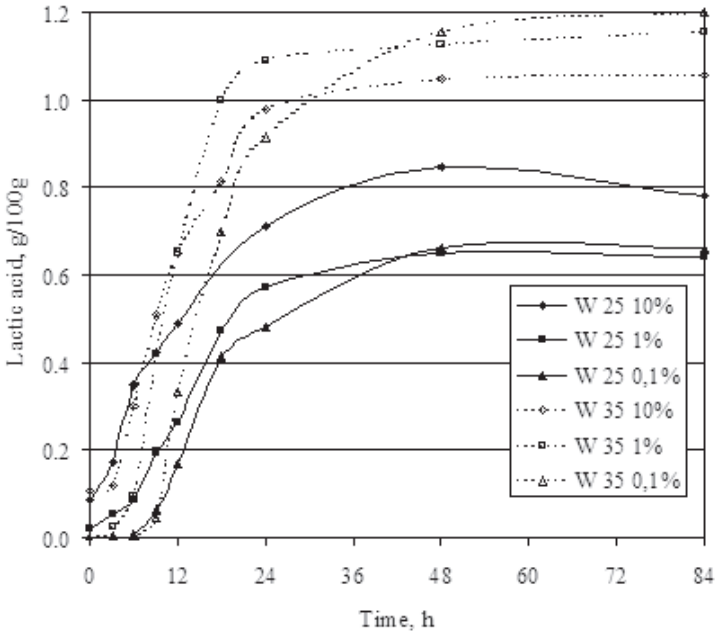

Figure 3: Lactic acid content in fermenting whole wheat slurry

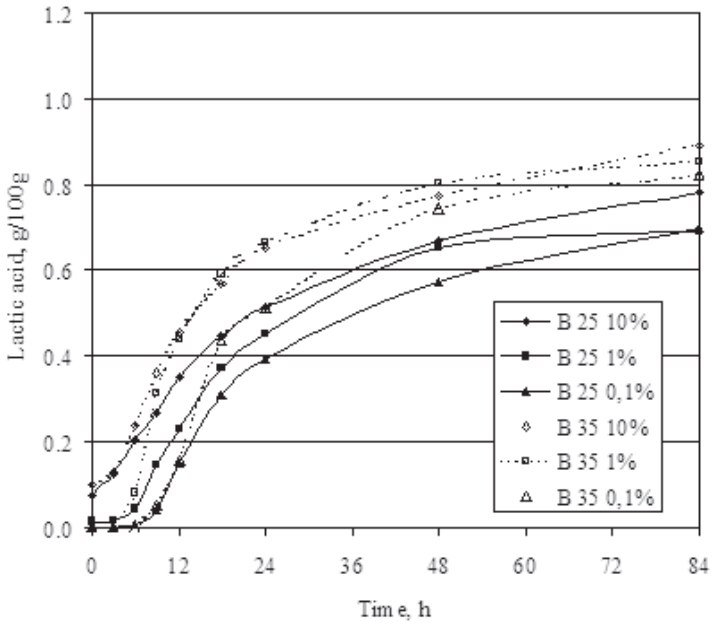

Figure 4: Lactic acid content in fermenting bagerivetemjöl slurry

pattern was similar, but not as accentuated, and the final amounts were all below 0,9 g per 100 g (Figure 4). Although the lactic acid amounts were not high, the pH reached using bagerivetemjöl was the lowest.

Lactic acid production rate

The production rate of lactic acid was higher at higher temperature in all cases. The results show a difference in buffering capacity for the different raw materials. For bagerivetemjöl a great portion of the outer parts of the grain are removed. In this case, only 70% of the grain are retained in the milling process of bagerivetemjöl. The production rate of lactic acid in the bagerivetemjöl slurry was lower that in the other slurries, but the change in pH was about the same (Figure 5).

The production rate of lactic acid was also influenced by the amount of inoculum. At 35°C, the initial production rate (just after the lag phase) was higher with 1% backslopping than with 10% backslopping. This can be seen in Figure 2, Figure 3 and Figure 4, where the curves for 1% backslopping closes in on, and after about 12 hours passes, the 10% backslopping curves.

Inoculum amount for backslopping

To test the importance of the amount of inoculum in the fermentation, three different amounts were used in the experiments. The original recipe's ladle was estimated to contain 100 g, which gave 10 g as normal inoculum for the 900-g laboratory

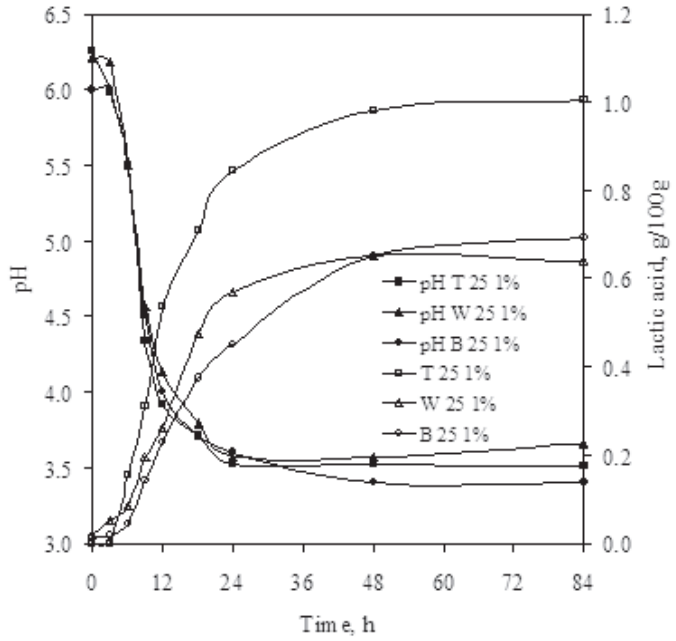

Figure 5. Lactic acid production and pH-development at 25°C, 1% backslopping, using tef, whole grain wheat and bagerivetemjöl.

scale slurry (approx. 1%). This amount was 10-folded and divided by 10, giving 1 g and 100 g respectively as the other assay inoculum amounts.

A higher amount of inoculum gave a quicker lowering of the pH, Figure 6 and Figure 7. This can be explained by the fact that the number of bacteria put into the slurry was higher, giving a higher lactic acid production. In addition, the initial pH was lower since the amount of inoculum put into the slurry was considerable. A 10% inoculum into the slurry can also be considered as a 10-folded dilution of the inoculum. This could theoretically mean a rising of the pH in the inoculum (approximately 3,5) with one unit (to approximately 4,5). In practise, the rising of the pH was higher. The bagerivetemjöl fermentation started at pH 4,8 while the flour of whole wheat and tef started at pH 5,4. At 35°C the 10% inoculum slurry had a short lag phase, while at 25°C the acid production started almost immediately.

A low inoculum (0,1%) gave a longer lag phase, and almost no change in initial pH compared to a slurry without inoculum.

Temperature dependence

All assays were made at two different temperatures: 25°C and 35°C.

Higher temperature raised the production rate of lactic acid. It also had an impact on the lactic acid bacteria in that the strains found were somewhat different.

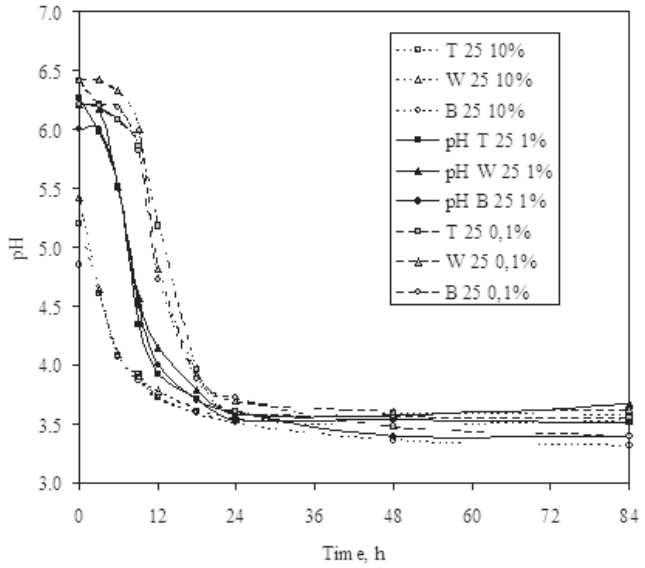

Figure 6: Development of pH at 25°C, using tef, whole grain wheat and bagerivetemjöl.

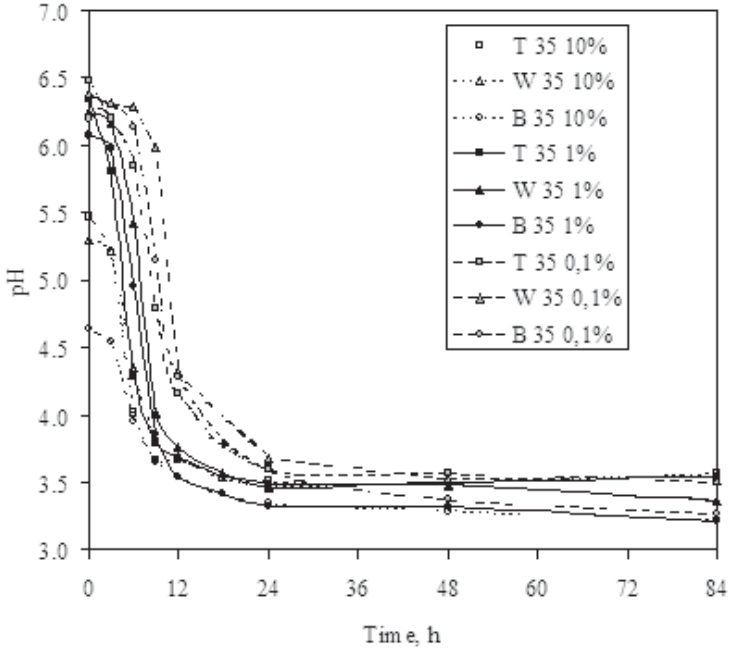

Figure 7. Development of pH at 35°C, using tef, whole grain wheat and bagerivetemjöl.

With a high inoculum, the temperature difference was not very important; the high starting number of lactic acid bacteria compensated for differences in temperature.

In whole grain wheat flour, there was a clear difference between the two temperatures used, the higher temperature giving higher concentrations of lactic acid. For tef the final difference was opposite, the final concentrations of lactic acid was higher at the lower temperature, but here the difference was much smaller.

Influence of raw material

Figure 8. Lactic acid content (total and undissociated) and pH-development at 25°C, 1% backslopping, using tef (T), whole grain wheat (W) and bagerivetemjöl (B) At 25°C and 1% backslopping the difference between the pH-development of the fermentations was very small (Figure 5), but the lactic acid production was very different with lowest production in bakery flour slurry and highest production in tef slurry. The food safety depends on the amount of undissociated lactic acid in the slurry. In Figure 8 it is shown that the concentration of undissociated lactic acid is rising much slower than the total amount. The pH has to go down to 4 before the concentration of undissociated lactic acid starts to rise significantly.

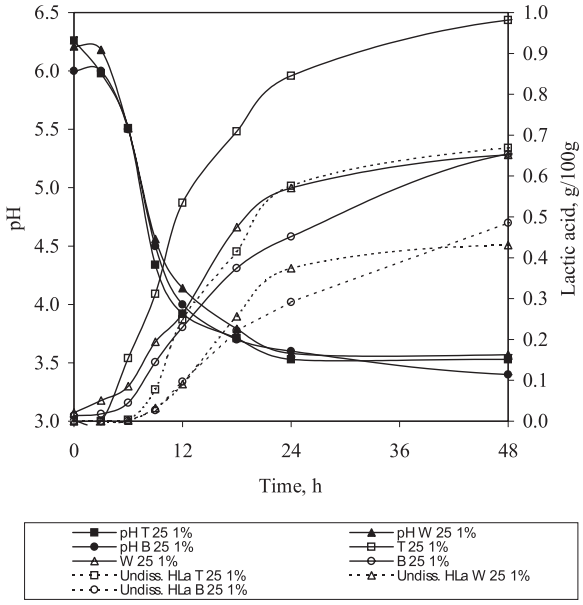

Figure 8: Lactic acid content (total and undissociated) and pH-development at 25°C, 1% backslopping, using tef (T), whole grain wheat (W) and bagerivetemjöl (B).

Titratable acidity

Table 1. Titratable acidity compared to HPLC-determination of lactic acid

The amounts of lactic acid derived from titrations corresponded to the values found using HPLC according to the results in Table 1. Titratable acidity gave higher values for lactic acid than HPLC-determination.

Table 1: Titratable acidity compared to HPLC-determination of lactic acid in slurry of bagerivetemjöl (B), whole wheat flour (W) and tef (T).

Cereal flour, backslopping amount	Temperature					
	25°C			35°C		
	Slope	Zero	R^2	Slope	Zero	R^2
B, 0,1%	1,1643	0,0574	0,9911	1,3160	0,0608	0,9877
B, 1%	1,1493	0,0772	0,9934	1,2357	0,0736	0,9956
B, 10%	1,1414	0,0669	0,9976	1,2267	0,0673	0,999
W, 0,1%	1,5619	0,0742	0,9842	1,2228	0,1039	0,9939
W, 1%	1,5317	0,1184	0,9835	1,1836	0,1209	0,9781
W, 10%	1,4097	0,1443	0,9859	1,2410	0,1489	0,9912
T, 0,1%	1,5301	0,0751	0,9838	1,5531	0,1190	0,9917
T, 1%	1,5439	0,0923	0,9927	1,4981	0,0976	0,9811
T, 10%	1,6212	0,0027	0,9698	1,5582	0,1083	0,9470

Relation pH – lactic acid

A combination of all the results regarding the relation between pH and lactic acid for the different raw materials (at all temperatures and back-slopping amounts) is given in Figure 9. The diagram shows that it was much easier to lower the pH in the bagerivetemjöl, and that tef flour was more resisting to changes in pH. At 25°C and with 1% back-slopping (normal injera production) the development of pH was very similar for the three different raw materials (Figure 5), although the lactic acid amounts were different with a much higher end concentration of lactic acid in the tef slurry.

Lactic acid bacteria at the final stage

The dominant species in the tef fermentation was *Lactobacillus plantarum* at a final level of approximately 10^8 cfu/g (Table 2). At 25°C only *L. plantarum* was found, of two different strains (1 and 2). Using the low amount of inoculum only 20% of the lactic acid bacteria belonged to strain 1, with normal inoculum amount 40% belonged to strain 1, and with high inoculum amount only strain 1 was present.

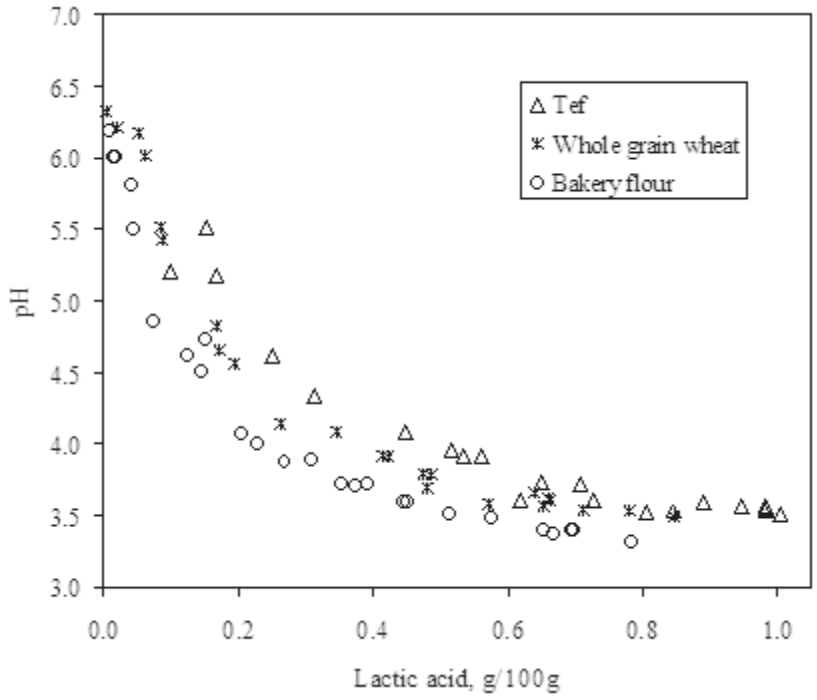

Figure 9: Lactic acid content and pH in fermenting slurry

Table 2. Lactobacilli count in final fermented tef slurry, cfu/g.

	Backslopping amount		
Temperature	0,1%	1%	10%
25°C	$2{,}2 \cdot 10^8$	$2{,}6 \cdot 10^8$	$2{,}6 \cdot 10^8$
35°C	$1{,}7 \cdot 10^8$	$1{,}0 \cdot 10^8$	$3{,}4 \cdot 10^8$

At the higher temperature (35°C), *L. plantarum* was also the dominant species, but other *Lactobacillus* species were also present. Low inoculum amount contained 40% *L. plantarum* strain 1. Three other strains were present, two were obligate homofermentative lactobacilli strain 4 and strain 5, while strain 6 seems to be an obligate heterofermentative lactobacilli. Normal amount of inoculum contained 80% of *L. plantarum*, 20% strain 1, 40% strain 2 and 20% strain 8. Additionally strain 7 was found that resembles strain 6 but not being completely identical. At high inoculum 40% of the isolates contained *L plantarum* (20% strain 1, 20% strain 2). The rest of the isolates contained obligate homofermentative lactobacilli of three different strains (strain 3, 4 and 5) that resemble each other genetically but have different phenotypes. These results are compiled in Table 3.

Table 3. Lactobacilli found in fermented tef slurry

		Temperature						
		25°C		35°C				
Bacteria		0,1%	1%	10%		0,1%	1%	10%
Lactobacillus	1 X	XX	XXXXX		XX	X	X	
plantarum	Strain 2	XXXX	XXX				XX	X
Strain	Strain 8						X	
Obligate	Strain 3							X
homo-	Strain 4					X		X
fermentative	Strain 5					X		X
Obligate	Strain 6					X		
hetero-f	Strain7						X	
ermentative								

Discussion

Lactic acid fermentation is a well-established method of treating foods. It is used as a household practise in many areas of the world. For most fermentations some kind of starter culture is used. This can be very simple, like using the same container as always, with its microbial flora on the inside walls. It can be made by using commercially produced starter culture specified for the fermentation. It can also be using a portion from the former fermentation made – backslopping.

Nout (1989) has shown that after a few backsloppings the system can be considered stable. In this trial, three consecutive backsloppings were made before the system was considered stable. The starting fermentation had a slow development as could be expected, while the second fermentation was very quick having active bacteria that has just entered the final static zone. The third fermentation was close to normal backslopping fermentation, and from the forth fermentation the behaviour was consistent.

A rise in the production rate of lactic acid will secure the food safety quicker, which is desirable under circumstances that other factors like taste and functional properties of the final food also will be considered.

The circumstances influence the development of the bacteria in the slurry. Inoculum amount, temperature and raw material influence the types of bacteria found in the fermentation.

Two different temperatures

The temperatures 25°C and 35°C were chosen for the experiments. The lower temperature was chosen since this is the temperature mainly found in Ethiopia. The high altitude at most parts of the country gives this comparatively low temperature for a tropical country, and it is also fairly stable around the year. The higher temperature was chosen, as it is a temperature that often can be found in tropical

countries during the hot time of the year.

The pH dropped quicker at the higher temperature.

At 25 C *Lactobacillus plantarum* was the only species found in the tef fermentation, while at the higher temperature other lactobacilli was also found. This indicates that the end product might be different, so even though a higher temperature can give a better food safety, the food item might not be exactly the expected.

Inoculum amount

A high inoculum amount gives a faster entry to the safe area where pathogenic bacteria can not survive. However, the high inoculum also changes the strains involved, and thus the changes on the product provoked by the bacteria will not be the same.

The optimum inoculum amount for fermentation needs to be established using sensoric criteria. The results of this study can be used to estimate the food safety during the process. A higher inoculum amount will make the entry into the safe area come quicker, but since the lactic acid bacteria strains involved changes, the final product will not be the same.

Lactic acid content

The lactic acid production rate at 35 °C was higher with the normal inoculum amount than with a high inoculum amount. This could be explained by the fact that these bacteria is continously in an acid environment, while the bacteria in a normal inoculum gets refreshed for every backslopping.

The content of lactic acid at a certain pH is very much dependent on the raw material.

Buffering effect

Due to buffering agents in the flours, the pH will rise more than expected at the backslopping. The different raw materials had different buffering capacity, indicating that they contained different amounts of buffering agents. The bagerivetemjöl had the lowest buffering capacity, while tef had the highest buffering capacity. An explanation to this can be that the outer parts of the grains contains compounds reacting as buffering agents. Tef has very small seeds which gives a high portion of outer parts, while in bagerivetemjöl the outer parts of the grain has been removed. The difference in buffering capacity is so big that to reach pH 4 you need more than the double amount of lactic acid in tef flour than in bagerivetemjöl.

With less buffering agents a low pH will be reached with less acid produced, and the low pH will then hindering the lactic acid bacteria to continue the production of lactic acid.

A rise in the production rate of lactic acid will secure the food safety quicker, which is desirable under circumstances that other factors like taste and functional properties of the final food also will be considered.

Effect of lactic acid on pathogenic organisms

Lactic acid inhibits many pathogenic bacteria, and the undissociated form of the acid is considered to be the active component (Robinson & Samona, 1992). The amount of undissociated lactic acid depends on both the concentration of lactic acid and the pH. The pKa of lactic acid is 3,86, giving that at pH=4,8 only 10% while at pH=3,86 half of the lactic acid is in the undissociated form (Figure 10). In contrast to the lactate ion, the uncharged undissociated form of lactic acid can penetrate the cell membrane of the bacteria. The cytoplasm of the bacteria has a much higher pH than the surrounding, which provokes the dissociation of the lactic acid molecule inside the bacteria, thus liberating H^+. This will lower the pH of the cytoplasm. The amount of undissociated lactic acid passing through the cell membrane will at a point near pH 4 be to high for the mechanisms in the cell dealing with the regulation of pH in the cytoplasm. The pH inside the bacteria will drop and eventually cause the destruction of the pathogen.

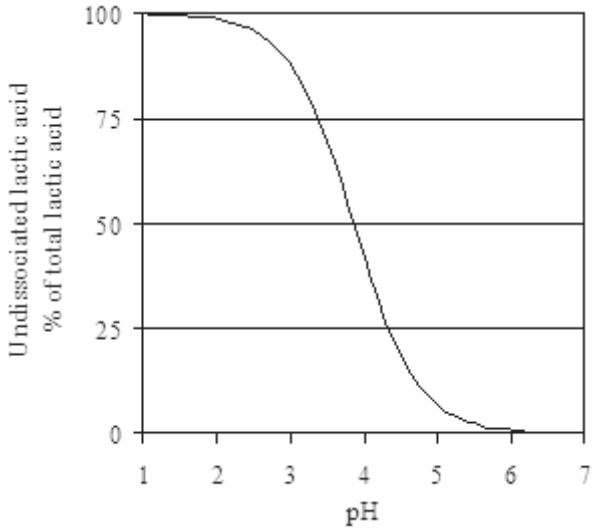

Figure 10: Portion undissociated lactic acid at different pH.

At the "normal" injera fermentation (tef 25°C 1%), it took twelve hours before the lactic acid content reached a level that gave some safety to the product, and another twelve hours to reach high levels of lactic acid (Figure 8). Therefore, the first twelve to twentyfour hours is the vulnerable period of the fermentation, where an infection by pathogenic bacteria can harm the safety of the product.

Regarding pathogenic bacteria, the final product in this type of fermentation, having its normal sourness, can be considered as a safe product (FDA 1992). No pathogens should be able to withstand the environment in the sour slurry for the length of time

involved in the process. If the vulnerable period is to be shortened, a solution could be to increase the inoculum amount. But this will probably also change the properties of the product.

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