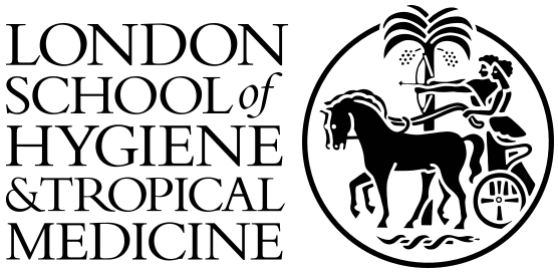

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The Microbiology of Teff (Eragrostis Tef) Enjera

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Abstract

Enjera, an indigenous Ethiopian pancake is the one of the staple foods of Ethiopians. There is few information available concerning the succession and activities of microflora of its fermentation. Therefore this study was carried out to assess the microbiology of teff enjera. A total of 34 samples from "kuncho" and "Magna" enjera batter were collected during 96 hr fermentation at 6 hr intervals."Kuncho" and "Magna" were bought from Debrezeit Agriculture Research Center and from Hawassa open market, respectively. Samples were analysed for changes in pH, titratable acidity (TA) and microbial count. The pH decreased with increasing TA during "kuncho" and "Magna" teff enjera batter fermentation. Total aerobic mesophilic count, LAB and yeast increasedby about 3 log cycles until 48 hr fermentation, while Enterobacteriaceae were reduced below detectable levels after 18 hr due to the low pH of the teff batter. Generally, the pH, TA and microbial count of enjera from the two cultivars of teff batter were not different.

Keywords: Enjera; Enterobacteriaceae; Lactic acid bacteria; Teff batter; Total aerobic mesophilic count; Yeast.

1. Introduction

Enjera is the most popular type of traditional bread, which is circular (about 45-60 cm diameter), thin, porous and pancake - like with numerous eyes. It has a sour taste due to natural fermentation. Enjera can be made from different cereals, including sorghum, teff, corn, wheat, barley, or a combination of some of these cereals. Enjera from teff (Eragrostis tef) is much more relish, by most Ethiopians, than that from any other source. Teff enjera is usually obtained after the flour of teff (Eragrostis tef) has been subjected to two stages of natural fermentation lasting for a total of 24 to 96 hours depending on the ambient temperature. The first stage of fermentation results in a liquid/solid separation, andit takes about two days. During the second stage of fermentation, the liquid layer is poured off and a boiled portion of the fermented dough known as absit in Amharic is added to the fermented batter and further incubated for at least 30 minutes to allow batter "rising" before baking commences. Besides sensorial characteristics, as flavour, aroma and color, fermentation improves the nutritional content of enjera, because it can decrease the relationships Iron: Phytates and Iron: Tannins complex (Annan *et al.* 2003).

A major source of inocula for teff fermentation is the teff flour itself, water and vessel. The traditional threshing processes of teff result in the contamination of the teff seeds with a very wide variety of soil and faecal material.

Today, the production of indigenous fermented foods and beverages is through spontaneous fermentation and backslopping (Achi, 2005). This is usually done by ecological control of starter microflora (Scott and Sullivan, 2008).Which is naturally selected as determined by the physico-chemical conditions of caring out of fermentation and present naturally in the raw materials or some products containing the desirable microbes from a previous fermentation (Scott and Sullivan, 2008).

Since enjera batter fermentation is based on natural inoculants, it is necessary to study the natural flora involved in its fermentation. Therefore, this study was aimed to assess the microbiology of teff (Eragrostis tef) enjera.

2. Materials and Methods

2.1 Teff Sampling

Two teff cultivars, "Kuncho" and "Magna" were bought from Debrezeit Agriculture Research Center and from Hawassa open market, respectively. Both varieties were cleaned of dust, other seeds and foreign matter. On the day of fermentation, both of the cultivars were separately ground into fine powder in a local flour mill before the start of batter preparation and fermentation.

2.2 Preparation and Fermentation of Teff Batter

Fermented teff batter was prepared under laboratory conditions. The fermentation was performed according to the traditional fermentation in households. It was initiated by sifting the flour and mixing 2 kg flour with 4 L water in fermenting plastic jar in a 1:2 ratio. Previously fermented dough (10 %) was then added to the mixture of flour and water to act as a starter and mixing thoroughly until homogenous slurry is obtained. The batter was equally distributed in 17 sample bottles and allowed to ferment for 96 hours at room temperature.

2.3 Sample Analysis

During the fermentation of teff enjera batter, aliquots of fermenting slurry in sample bottles were aseptically withdrawn at 6 hours intervals and analysed for pH, titratable acidity (TA) and microbiological changes. Altogether, 34 samples (17 from "Kuncho" and 17 from "Magna") were analyzed.

2.3.1 *Change in pH*

The change in pH as the fermentations progressed was measured using a BASIC 20 pH meter (Crison Instruments, S. A. Riera Principal, 34-36.08328 Alella, Spain) after calibrating with standard buffers (Hamiltun Duracal Buffer, Switzerland) of pH 4 and 7 at 25^0C.

2.3.2 *Total Titratable Acidity*

The total titratable acidity was determined by titrating 10 ml of fermentation aliquot against 0.1 N NaOH to pH 8.30, using phenolphthalein as indicator. The total acid content of the sample was determined as the percentage of lactic acid as follows:

$$\% \text{ Lactic acid} = \frac{\text{ml of 0.1 molar NaOH} \times 0.9 \times 100}{\text{Volume of sample}}$$

Where the lactic acid equivalent factor is 0.9 (Katinaa et al., 2007)

2.3.3 Microbiological Analysis

One bottle of teff batter (200ml) was taken from each fermenting teff cultivar with in 6 hr intervals for microbiological analysis. Ten ml of batter were sampled under aseptic conditions. The sample was homogenized with 90 ml sterile 0.1% peptone water and appropriate serial dilutions were made by the method of Yousef and Carlstrom (2003) to obtain countable numbers of colonies on appropriate agar plates.

2.3.3.1 Total Aerobic Mesophilic Count

Total aerobic mesophilic count were enumerated by spread plating 0.1 ml of the diluted sample on pre-dried surface of plate count agar (PCA,HIMEDA M091), after having incubated at 30^0C for 48 h.

2.3.3.2. Enumeration of Enterobacteriaceae

Enterobacteriaceae were enumerated by spread plating 0.1 ml of the diluted sample on pre-dried surface of violet red bile dextrose (VRBD) agar (CDH JO 0003). Purple red colonies on VRBD agar plates were counted as members of Enterobacteriaceae after incubate at 30^0C for 24 hr.

2.3.3.3 Lactic Acid Bacteria

Lactic acid bacteria (LAB) were enumerated by spread plating 0.1 ml of the diluted sample on pre-dried surface of De Man Rogas Sharpe (MRS) agar (OXOID CM 0361) plates after anaerobic incubation at 30^0C for 48 to 72 hr.

2.3.3.4 Yeast

Yeasts were counted by spread plating 0.1 ml of the diluted sample on pre-dried surface of yeast extract glucose chloramphenicol (YGC) agar, which is consisted of (gram per L): yeast extract, 5.0; glucose, 20.0; chloramphenicol, 0.1;

bromophenol blue, 0.01; agar 15; pH, 6.0 to 6.2 after incubation at 28^0C for 3 to 5 days.

2.3.4 Organoleptic Analysis

Effect of fermentation time and type of teff cultivar on sensory attributes of enjera were assessed using consumer-oriented sensory panel. Eight (8) types of enjera were produced at 24, 48, 72 and 96 h fermentation time from "Kuncho" and "Magna" teff cultivars were evaluated by a panel of consumer-oriented assessors. The panelists were asked to rate samples for taste, aroma, topside appearance (eyes), backside appearance ("Sebekt") and texture, and to score from 1to 5 (where 1 = poor, 2 = fair, 3 = satisfactory, 4 = very good and 5 = excellent quality). Samples were presented on identical serving trays and coded with three digit random numbers. The order of sample presentation was randomized. Clean water held at room temperature was served for cleansing mouth palate of assessors before and between testing of enjera samples. Samples of enjera being evaluated were expectorated into prepared beaker.

2.3.5 Statistical Analysis

Microbiological counts were normalized by Log10 transformation. Differences between means of sensory scores of enjera (analyzed between teff cultivar and fermentation time) were analyzed using SPSS version 16.0. One way analysis of variance was performed for determination of individual differences between 8 treatments.

3. Result

3.1 Changes in pH and Titratable Acidity occurred during Teff Batter Fermentation

The changes in pH and titratable acidity were monitored during traditional fermentation of teff batter from "kuncho" and "Magna" at 6 hours interval for 96 hours. The pH decreased from an initial pH 6.65 to 3.49 during fermentation of "kuncho" teff batter and from pH of 6.66 to 3.44 during fermentation of "Magna" teff batter. The titratable acidity increased from 0.1 to 1.32 during fermentation of "kuncho" teff batter and from 0.10 to 1.39 during fermentation of "Magna" teff batter (Figure 1).

3.2 Microbial Changes Occurred during Teff Batter Fermentation

The microbial changes during the traditional fermentation of teff batter from "Kuncho" and "Magna" flour were onitored. The total aerobic mesophilic counts (AMC) were high (cfu/ml) and continued to increase steadily until 48 hr of fermentation of enjera batter with the LAB and yeasts being the predominant microorganism (Figure 2). The count of Enterobacteriaceae decreased to non-detectable levels after 18 hr of fermentation. The total aerobic mesophilic count ranged between 5.1 to 8.46, the lactic acid bacteria 5.12 to 8.47, the yeast 5.02 to 8.45 and Enterobacteriaceae 4.17 to <1cfu/ml during fermentation of "Kuncho" teff batter (Figure 2a). The total aerobic mesophilic count ranged between 5,15 to 8.42, the lactic acid bacteria 5.15 to 8.46, the yeast 5.06 to 8.2 and Enterobacteriaceae 4.20 to <1cfu/ml during fermentation of "Magna" teff batter (Figure 2b).

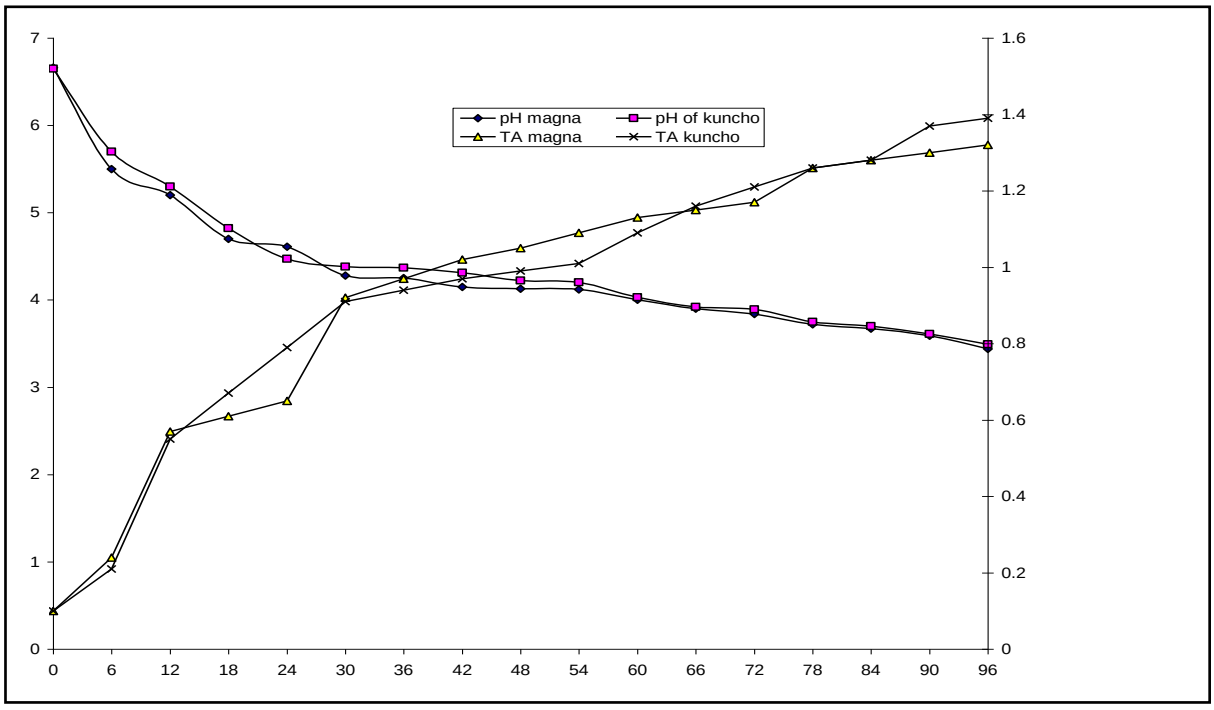

Figure 1. Change in pH and TA during fermentation of teff *enjera* batter.

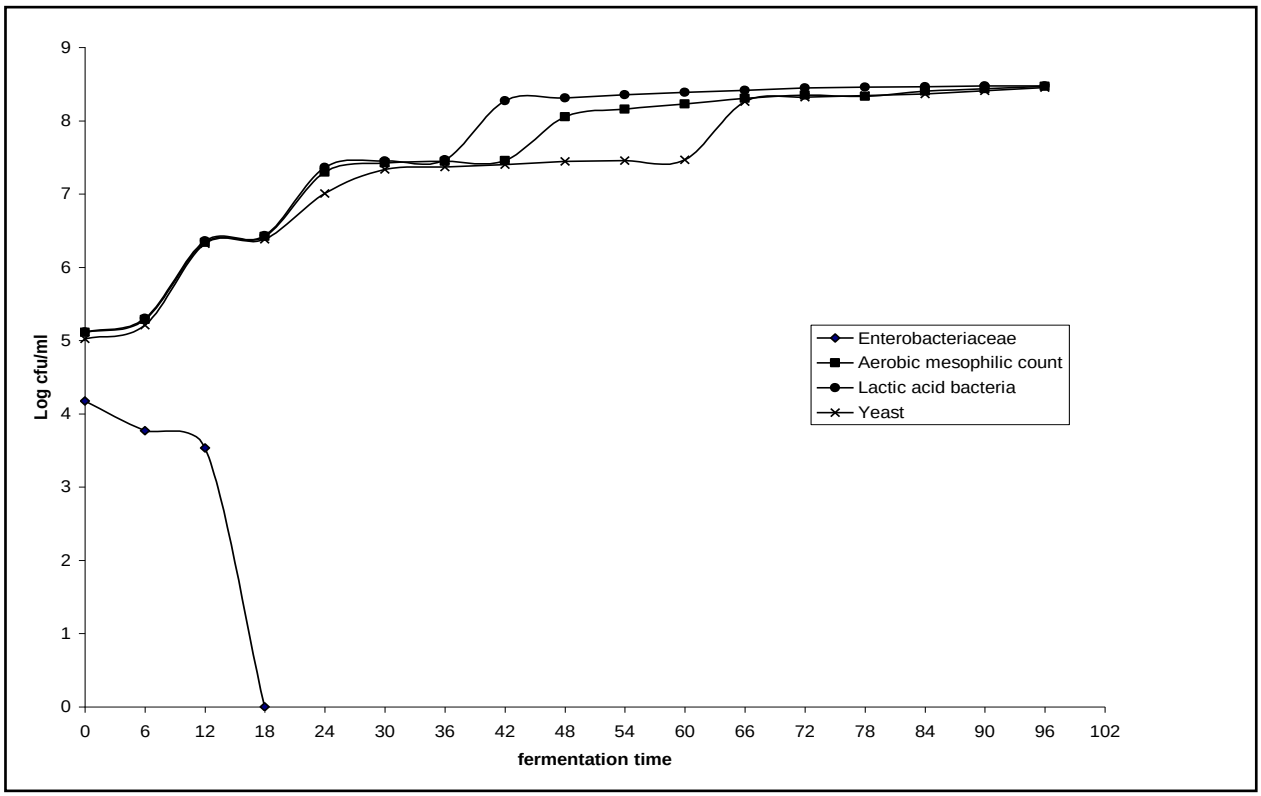

Figure 2a. Microbial count during *enjera* batter fermentation from "Kuncho" teff flour.

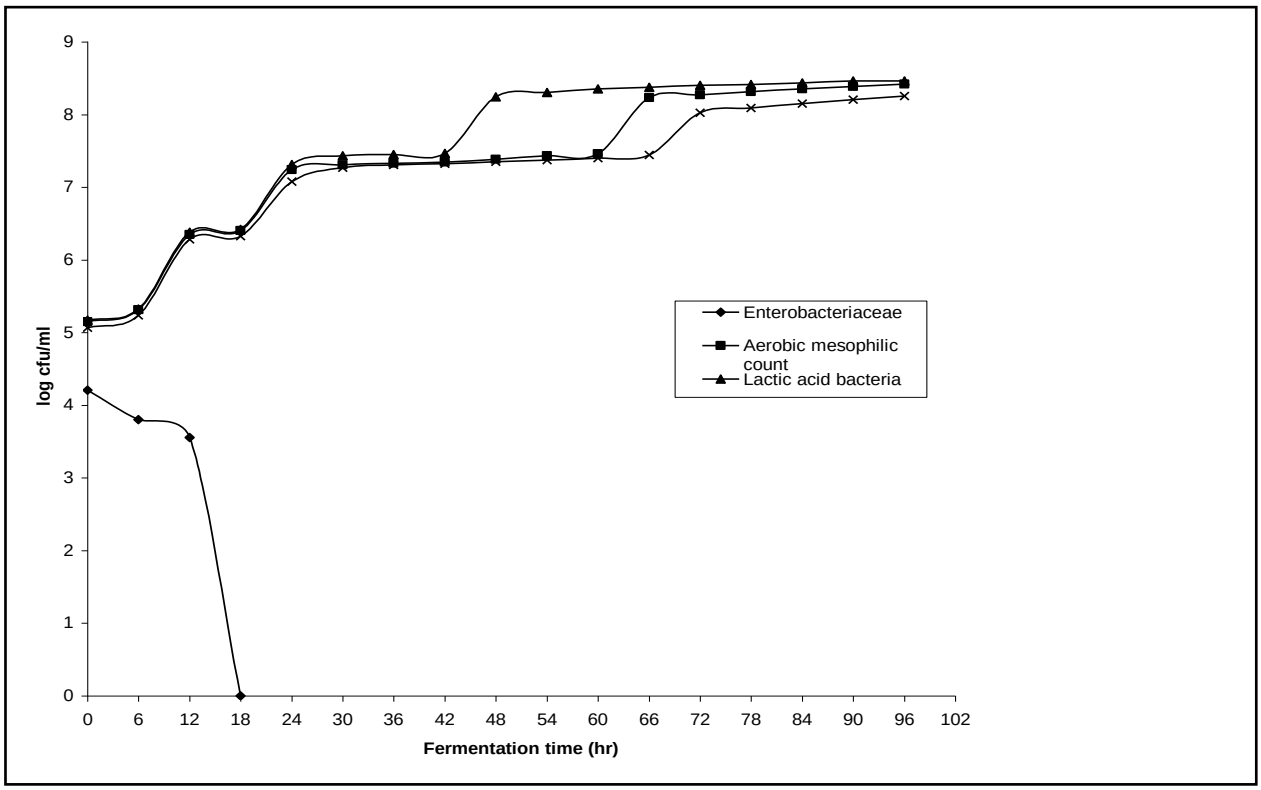

Figure 2b. Microbial count during *enjera* batter fermentation from "Magna" teff flour.

3.3 Sensory Evaluation

The final products (enjera) produced from 24,48,72,96 hr fermented batter of "Kuncho" and "Magna" teff were served to consumer-oriented judges. The sensory scores to taste and aroma to all samples were more than satisfactory to the sensory evaluation panel (score >3) and did not differ significantly ($p<0.05$) (Table 1).

Table 1. The Mean (±S.D.) sensory scores of *enjera* with different duration of fermentation Vs teff cultivar.

Teff cultivar	Fermentation time(hr)	The mean scores of sensory attributes of *enjera*[*]				
		Taste	TS app.	BS app.	Texture	**Aroma**
"Kuncho"	24	3.1±1.15[a]	3.1±0.89[abc]	3.1±0.84[a]	3.0±0.63[a]	**3.2±0.98 [a]**
	48	3.0±0.97 [a]	2.5±0.89[c]	2.8±0.66[a]	2.9±0.68[a]	**3.8±0.93 [a]**
	72	3.2±1.05 [a]	2.8±1.13[abc]	2.7±0.95[a]	2.9±0.99[a]	**3.3±1.06 [a]**
	96	3.4±1.21 [a]	2.9±1.03[abc]	3.1±0.85[a]	3.2±0.91[ab]	**3.4±0.72 [a]**
"Magna"	24	3.6±1.21 [a]	3.4±0.73[b]	4.1±0.61[b]	3.8±0.58[c]	**3.8±0.58 [a]**
	48	3.5±1.21 [a]	3.3±1.07[b]	3.6±1.03[b]	3.6±1.03[c]	**3.8±0.86 [a]**
	72	3.4±1.09 [a]	3.1±0.99[abc]	4.1±0.62[b]	3.4±0.89[bc]	**3.4±0.96 [a]**
	96	3.1±0.93 [a]	3.0±1.21[abc]	3.9±0.81[b]	3.1±0.99[b]	**3.4±0.89 [a]**

[*]**1 = poor, 2 = fair, 3 = satisfactory, 4 = very good and 5 = excellent quality of *enjera*, means with different letters in the same column indicates the presence of significant difference (p<0.05).**

4. Discussions

"Kuncho" and "Magna" teff cultivars were used in traditional enjera batter fermentation. The changes in pH, TA and microbial count were monitored during enjera batter fermentation (Figure 1and Figure 2). Similar fermentation progress was observed in both types of teff batter. The pH declined steadily with increasing total TA during "Kuncho" and "Magna" batter fermentation due to the presence of LAB throughout fermentation time. The decrease in pH and increase in TA followed the same trend as reported for other traditionally fermented foods such as in fermentation of borde (Kebede et al. , 2002), Kunun-zaki (Agarry et al., 2010), Soybeans (Tinna A *et al* 2015).

There were increases in numbers of AMC, LAB and yeast. The AMC were high and continued to increase steadily until 48 hr fermentation of enjera batter with the LAB and yeasts being the predominant microorganism in the batter. AMC, LAB and yeast increased by about 3 log cycles, Enterobacteriaceae decreased to non-detectable levels after 18 hr of fermentation possibly due to the low pH of enjera batter (Figure 2). Decreasing of Enterobacteriaceae in low pH was also shown in fermenting teff slurry (Peter, 1999). The author found that the growth of E. coli is significantly reduced to non-detectable levels around pH 4.0. The largest increase in the numbers of AMC, LAB and yeasts were noted during the 48 hr of fermentation. The results obtained in the present study are in close agreement with those reported for sourdoughs by Vincenzo *etal* (2005). As reported in the fermentation of other indigenous Ethiopian fermented foods, the Enterobacteriaceae could participate in the initiation of fermentation and reduced as subsequent acid formation by LAB increased(Ekwem , 2014). Acid production together with the formation of antimicrobial components determines the microbial stability. There were no difference between "Kuncho" and "Magna" in microbial load. This may reflect that the type of cultivar may not contribute for source or growth of microorganisms, but the contamination source and handling of teff grains.

The effect of teff cultivar and duration of fermentation were assessed (Table 1). Significant variation (p<0.05) was found in the scores to topside appearance ("eye"), backside appearance ("sebeket") and texture. For all the tested parameters, enjera prepared from "Magna" batter fermented for 24 hr has the best quality and more than satisfactory to all sensory attributes followed by enjera prepared from "Magna" batter fermented for 48 hr. This could be because of color of the teff and duration of fermentation. Long fermentation time make the taste and aroma of enjera more sour.

5. Conclusion

This study has shown that the changes in microbial count (AMC, Enterobacteriaceae, LAB and yeasts), pH and TA during traditional fermentation of "Kuncho" and "Magna" teff enjera batter for 96 hr. The pH decreased with increasing TA during the teff batter fermentation associated with increasing in LAB count. The AMC, LAB and yeasts were high and continued to increase steadily until 48 hr of fermentation of enjera batter by about 3 log cycles. Enterobacteriaceae decreased to non-detectable levels after 18 hr fermentation due to the low pH of enjera batter as result of acid production

mainly by LAB.

The effects of teff cultivar and duration of fermentation on sensory quality of enjera was assessed. The sensory scores in taste and aroma to all treatment and control enjera were more than satisfactory (score >3) and did not differ significantly ($p<0.05$). However the interaction between cultivars and duration of fermentation differed significantly ($p>0.05$) in score for appearance and texture of enjera. Change in pH, TA and microbial count during fermentation of teff batter is shown the same trend.

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