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Microbiology of *siljo*, a traditional Ethiopian fermented legume product

T. Mehari and M. Ashenafi*

Siljo was prepared by thoroughly cooking powdered horsebean (*Vicia faba*) in safflower (*Carthamus tinctorius*) extract into a semi-solid slurry and then adding to it untreated black mustard (*Brassica nigra*) powder after cooling to 50°C. The black mustard powder was the source of starter microorganisms, with *Lactobacillus acidophilus*, *L. plantarum* and *L. delbruekii* initiating and later dominating the fermentation process. The pH of the fermenting mass dropped to 4.5 within 36 h and reached 4.0 at 168 h. Aerobic mesophilic bacteria and lactic acid bacteria were each present at about 1×10^{10} c.f.u./ml after 36 h of fermentation but *Enterobacteriaceae* were never detected. The dominant aerobic mesophilic flora consisted of *Micrococcus*, *Bacillus* and *Lactobacillus* spp. Crude protein, crude fat and ash increased slightly during the fermentation, with final values of around 28%, 25% and 7%, respectively, but there was a marked increase in protein availability and concentration during the fermentation.

Key words: Fermented foods, microbial flora, nutritional quality, *siljo*.

The diet of the average Ethiopian consists of only a limited number of foods. For the most part, these are based on cereals, supplemented, in certain cases, by a stew made mainly of pulses. Although the protein content of these legumes is about 20% to 25%, the traditional processing involved in preparing the stew decreases the protein content down to 14% to 17% (Agren *et al.* 1975). In a previous study it was demonstrated that a *tempeh*-type fermentation of horsebean could increase soluble proteins by 60% (Ashenafi & Busse 1991a). There are only a few studies on the microbiology of some Ethiopian fermented foods (Gashe *et al.* 1982; Gifawossen & Bisrat 1982; Gashe 1985, 1987; Ashenafi 1994). There is no information available on the microbiology or nutritional quality of *siljo*. *Siljo* is a fermented product made from safflower (*Carthamus tinctorius*) extract and horsebean (*Vicia faba*) flour. It is a popular food in the central highlands of Ethiopia during the fasting period before Easter. Its fermentation is spontaneous and it is usually ready for consumption after 3 days. The fermented product is a greyish gruel with a typical acidic and

mustard flavour. It is consumed as a side dish to any one of the major legume-based sauces along with *injerra*, a traditional pancake. *Siljo* is believed to add some variety to the otherwise monotonous fasting dishes of the average highland Ethiopian. As a household product, it is not produced in large amounts and whatever is produced is consumed within 2 or 3 days. The purpose of the present study was to evaluate some of the microbiological and nutritional properties of fermenting *siljo*.

Materials and Methods

Preparation of Siljo
Siljo was prepared following traditional methods (Figure 1). Powdered horsebean was thoroughly mixed with safflower extract and cooked well to a gruel consistency. This was cooled to about 55°C and black mustard powder, homogenized in warm water, was then added to it. After a thorough mixing, the gruel was allowed to ferment at room temperature. At around 32 h, peeled garlic and rue (*Ruta chalepensis*) leaves were washed and added to it. *Siljo* is usually ready for consumption after 62 h of fermentation.

Microbiological Analysis of Samples
Fermenting *siljo* (10 ml) was homogenized in 90 ml sterile water and examined microbiologically. Aerobic mesophilic bacteria were

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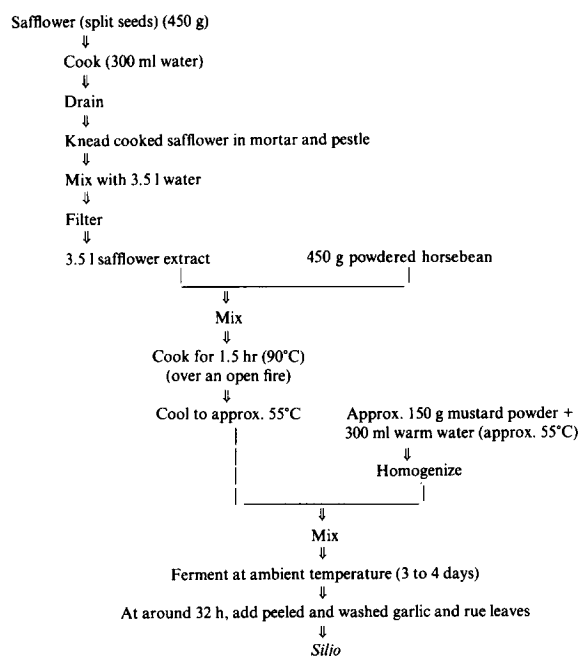
Safflower (split seeds) (450 g)
⇓
Cook (300 ml water)
⇓
Drain
⇓
Knead cooked safflower in mortar and pestle
⇓
Mix with 3.5 l water
⇓
Filter
⇓
3.5 l safflower extract   450 g powdered horsebean
|_____|
⇓
Mix
⇓
Cook for 1.5 hr (90°C)
(over an open fire)
⇓
Cool to approx. 55°C   Approx. 150 g mustard powder +
| 300 ml warm water (approx. 55°C)
| ⇓
| Homogenize
|_____|
⇓
Mix
⇓
Ferment at ambient temperature (3 to 4 days)
⇓
At around 32 h, add peeled and washed garlic and rue leaves
⇓
Siljo

Figure 1. *Siljo* fermentation process.

counted on standard plate-count agar (PC; Merck) after incubation at 30 to 32°C for 48 h. *Enterobacteriaceae* were enumerated on violet red bile glucose (VRBG) agar (Oxoid) plates after incubation at 30 to 32°C for 24 h, the purple-red colonies being considered *Enterobacteriaceae*. Enumeration of lactic acid bacteria was done on MRS agar (Oxoid) plates. Colonies were counted after incubation in an anaerobic jar (Oxoid) at 32°C for 48 h.

Yeasts and moulds were counted on chloramphenicol/ Bromophenol-Blue agar (CBB) containing (g/l): yeast extract, 5.0; glucose, 20.0; chloramphenicol, 0.1; Bromophenol Blue, 0.01; and agar, 15; at pH 6.0 to 6.4. Colonies were counted after incubation at 25 to 27°C for 5 days.

Flora Assessment: After colony counting, 20 colonies were picked at random from countable PC agar plates and identified by cell morphology and motility, both studied by phase-contrast microscopy, and by Gram reaction (Gregersen 1978), cyto-chrome oxidase (Kovacs 1956), glucose metabolism (Hugh & Leifson 1953) and catalase tests with 3% (v/v) H_2O_2 solution. Catalase negative and non-sporing rods and cocci, isolated from MRS plates, were considered as lactic acid bacteria and were further characterized according to Cowan & Steel (1974). Yeast isolates were characterized according to the methods of Farkas (1984) and by the auxanographic method of Barnett *et al.* (1979).

Moisture Content
Moisture content was determined by drying a sample to constant weight in an oven.

Biochemical Analyses
Raw powdered horsebean and fermenting *siljo* samples were analysed for total crude protein, crude fat and ash (Anon. 1990). *In vitro* protein availability was estimated as described by Kazanas & Fields (1981). Native SDS-PAGE was performed by the method

of Fling & Gregerson (1981), with a 10% to 25% linear gradient, and protein concentration was determined using the Bradford method.

Results

The cooked bean flour slurry had a low microbial load whereas the black mustard powder and the rue leaves, in contrast, had high microbial counts. The lactic acid flora consisted of *Lactobacillus* spp. The microflora of the cooked bean slurry was dominated by *Bacillus* and *Micrococcus* spp. whereas that of the black mustard powder consisted mainly of *Lactobacillus* spp., *Enterobacteriaceae* and *Bacillus* spp. (Table 1).

Counts of aerobic mesophilic and lactic acid bacteria increased and the pH dropped during the fermentation process. High counts were maintained from 36 to 192 h. The pH was ≤4.5 after 36 h of fermentation. *Enterobacteriaceae* were not detectable after 12 h fermentation. Yeasts and moulds were first detected immediately after the introduction of the rue leaves and persisted until 72 h (data not given). The fermentation process was mainly dominated by *Micrococcus*, *Lactobacillus* and *Bacillus*. *Lactobacillus* spp. markedly dominated the flora after 36 h whereas *Bacillus* spp. remained as part of the dominant flora without showing a noticeable increase during the fermentation time (Table 2).

Black mustard powder contained *L. acidophilus*, *L. del-bruekii* and *L. plantarum*, and the yeasts *Saccharomyces cerevisieae*, *Rhodotorula glutinis*, *Yarrowia lipolytica* and *S. rouxii*. The fermentation, however, was dominated by *L. plantarum* and *L. acidophilus*.

The moisture content of *siljo* was about 86%. A slight increase in crude protein and fat was observed throughout the fermentation (Table 3) but there was a marked increase in protein availability as fermentation progressed and this was accompanied by an increase in protein concentration in the soluble fractions of the papain-treated samples.

Discussion

The major constituents of *siljo* are safflower extract and powdered horsebean. However, the thorough cooking of these constituents results in the elimination of the active fermenting microorganisms, such as lactic acid bacteria or *Enterobacteriaceae*, which are usually important in the fermentation of various traditional Ethiopian food products (Gashe *et al.* 1982; Gashe 1985, 1987; Ashenafi 1994). The two major groups of bacteria which were isolated from the cooked bean slurry, namely *Micrococcus* and *Bacillus* species, had either been introduced from the mixing utensils used to cool down the slurry before the introduction of the black mustard powder or had survived the cooking

Table 1. Microbial counts and dominant flora of *siljo* raw materials.

	Aerobic mesophils (log c.f.u./g)	No. of isolates	No. of individual isolates*					
			M	L	B	E	A	Y
Cooked bean powder	2.30	40	17	–	23	–	–	–
Black mustard powder	6.05	60	2	11	19	16	5	7
Rue leaves	4.20	ND						

*M—*Micrococcus*; L—*Lactobacillus*; B—*Bacillus*; E—*Enterobacteriaceae*; A—*Acintobacter*; Y—*Yeasts*. ND—Not determined.

Table 2. Microbial counts of fermenting *siljo*.

Time (h)	pH	Counts (log c.f.u./ml)		No. of individual isolates from AMC plates*				
		AMC	LAB	M	L	B	Ac	Al
0	6.0	4.77	1.78	28	–	4	8	–
12	6.0	6.46	5.34	10	6	14	6	2
24	5.5	8.32	7.15					
36	4.5	9.42	9.31	5	22	13	–	–
48	4.5	10.08	9.98	3	26	11	–	–
60	4.2	10.34	10.26	3	26	11	–	–
72	4.2	10.58	10.53	2	25	13	–	–
96	4.2	10.72	10.69	3	25	12	–	–
120	4.1	10.88	10.82	8	19	13	–	–
144	4.1	10.53	10.50	10	20	6	4	–
168	4.0	10.04	9.91	13	16	7	4	–
192	3.9	9.23	9.16	10	14	8	–	8

*40 isolates were taken from each plate: M—*Micrococcus*; L—*Lactobacillus*; B—*Bacillus*; Ac—*Acintobacter*; Al—*Alcaligenes*. AMC—Aerobic mesophilic count; LAB—Lactic acid bacteria.

Table 3. Changes in some nutritional properties of fermenting *siljo*.

Time (h)	Crude protein (%)	Crude fat (%)	Ash (%)	Protein availability (%)			Protein concentration (%)
				With papain	Without papain	Difference	
0	27	21	7	1.36	0.25	1.10	1.34
24	27	20	7	1.80	0.40	1.40	1.47
48	28	23	7	2.25	0.65	1.60	1.72
72	28	25	7	4.60	1.62	2.98	3.01
96	28	25	7	4.91	1.50	3.41	3.22

temperature. Although black mustard powder is added to impart desirable flavour to the fermented product, this study has shown that it may be considered as the main source of starter microorganisms for *siljo* fermentation. *Siljo* fermentation is basically an acid fermentation and requires active, acid-producing *Lactobacillus* spp. which, in this case, were obtained from the black mustard powder. The black mustard also contained a variety of yeast strains but yeasts were only detected in the fermentation when rue was added and may not play any important role in the fermentation. Rue leaves, added for flavour, contained a substantial number of bacteria and yeasts but may not be considered

as a source of starter microorganisms because they were added after active fermentation had started and the pH had dropped to 4.5. In fact, the leaves may be considered as source of undesirable microorganisms. *Alcaligenes* spp. which appeared in the later stages of the fermentation, may be important in subsequent product spoilage or spoilage during cold storage.

The *Enterobacteriaceae* were below detectable levels throughout the fermentation and this could be due to the initial heat treatment and the production of various acids and metabolites by the lactobacilli which are inhibitory to many Gram-negative rods. Since yeasts and moulds can

grow at low pH, their disappearance after 72 h may be due to inhibitory metabolites produced by the competing microorganisms. *Bacillus* isolates constituted the dominant flora during the fermentation but these could perhaps be occurring as spores and were probably not active in the *siljo* fermentation because *Bacillus* spp. do not multiply at low pH (Ashenafi & Busse 1991b). *Bacillus* spp. may be important in the fermentation of a variety of legumes in West Africa, where the pH does not drop low enough to inhibit their multiplication (Ogabdu & Okagbue 1988; Jidiani & Okeke 1991; Achi 1992; Nwosu & Ojimelukwe 1993).

The starters for *siljo* fermentation in this study were considered to be *L. acidophilus*, *L. delbruekii* and *L. plantarum*. Further experiments are, however, required to demonstrate if pure lactic cultures could result in a typical and acceptable *siljo*. In addition to producing acids and other flavour components, *Lactobacillus* spp. are known to have antibacterial activities (Lindgren & Clevstroem 1978) and they produce a variety of bacteriocins which could control the growth of undesirable microorganisms (Tagg *et al.* 1976). *Lactobacillus plantarum* is important in vegetable fermentation (Vaughn 1985) and can inhibit a variety of food-borne pathogens during *tempeh*-type fermentations of horsebean and other legumes (Ashenafi 1991; Ashenafi & Busse 1991a, 1992) and other food fermentations (Gilliland & Speck 1972). Milk fermented with *L. acidophilus* is believed to have medicinal value (Gilliland & Speck 1977).

During the fermentation process, more amino acids became available and soluble. By mixing a sample in water and filtering it, protein and/or carbohydrate solubilized by the enzymes of the microorganisms can be determined. Using this technique, only 1.1% more dry matter was lost in the non-fermented samples treated with papain than the non-fermented samples without papain. The loss of dry matter due to papain digestion of the fermenting mass gradually decreased as fermentation progressed. This remarkable increase in soluble proteins may be very important in the improvement of the diets of the average Ethiopian. Since animal products are not affordable by most people in the developing world, and since plant proteins are generally considered to be less available to humans, the high level of water-soluble protein due to *siljo*-type fermentation of legumes is of a significant importance in improving the nutritional availability of these macromolecules.

Acknowledgements

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