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MICROBIOLOGICAL STUDY OF WAKALIM, A TRADITIONAL ETHIOPIAN FERMENTED SAUSAGE

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ABSTRACT: *Wakalim* is a traditional Ethiopian fermented beef sausage prepared and consumed commonly in Harar, Eastern Ethiopia. It has good keeping quality and the traditional processing technique is applicable at the household level. In this study, detailed information pertaining to the preparation processes and the raw materials used for its preparation was documented. Moreover, the safety quality of the ready-to-eat product was evaluated through microbiological analysis of the product as it was availed to the local consumers. The raw materials required for preparation of *wakalim* consist of lean meat (70%), fat (5%), salt (2%), garlic (1%), onion (17%) and other spices (5%). *Wakalim* samples were found to be dominated by aerobic mesophilic bacteria (AMB), mainly aerobic spore formers (ASF), followed by lactic acid bacteria (LAB) and *staphylococci*, with mean counts (log cfu/g) of 6.02, 4.70, and 4.59, respectively. Species of LAB isolated from w*akalim* samples mainly consisted of *Pediococcus pentosaceus*1 (29%), *Lactobacillus plantarum*1 (19%), *Ped. pentosaceus*2 (17%), *Lb. brevis*1 (16%), *Lactococcus lactis ssp. lactis* (6%), *Lb. pentosus* (4%), *Lb. brevis*3 (3%), and other *Lactobacillus* and *Pediococcus* species (6%). The average pH and titratable acidity of *wakalim* samples were 5.35 and 6.4%, respectively.

Key words/phrases: Ethiopia, Harari, LAB, Traditional fermented sausage, Wakalim

INTRODUCTION

Meat is a nutritious food for humans, and at the same time, an ideal growth medium for many microorganisms because of its high moisture content, rich nitrogenous compounds, minerals and other growth factors. Thus, unprocessed fresh meat is a highly perishable product. Among the factors that influence the keeping quality of meat are holding temperature, atmospheric oxygen, endogenous enzymes, moisture, light and, most important, microorganisms (Serdengecti *et al.*, 2006).

In response to problems associated with limited keeping quality of fresh meat and other animal and plant products, a number of food preservative

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techniques, such as drying, salting, fermentation, refrigeration, or freezing, evolved during the last 5,000 to 10,000 years (IOM, 1985). Sausage making, thus, evolved as an effort to economize and preserve meat that might not be consumed fresh at slaughter,

Fermented sausages are essential parts of diets in all regions of the world with an estimated average consumption rate of 180,000 metric tons per year in Great Britain alone (Anon, 2000). They also account for 42% share of all fermented meat products in Turkey (Erdo-Rul *et al.*, 2002). In the French meat industry, the annual production of dry fermented sausages was about 100,000 tons (Lebert *et al*., 2006). In Germany alone, 33.5 kg sausages were consumed annually per person (Bremer *et al.*, 2004). The annual production of *Chorizo* and *Salchichon,* the two most popular varieties of Spanish sausages, was estimated at 97,000 and 35,000 tons/year, respectively (Gonzalez-Fandos *et al*., 1999). According to Rantsiou and Cocolin (2006), extensive microbiological studies have been made on traditional sausages produced in different parts of the world including Greece, Italy, Spain, France, Turkey and Argentinia.

Wakalim is a highly-spiced traditional Ethiopian fermented beef sausage. Traditionally, its preparation relies on spontaneous fermentation and ingredients are the chief sources of fermenting microorganisms. It is an indigenous fermented product made mainly from meat and different spices. It is favored for its good keeping quality and applicability of the technique at the community level. Its preparation is a four-step process that includes the preparation of a casing called '*merechi*', mincing of meat, stuffing, and fermentation.

Although there is some information on social aspects of *wakalim* production and consumption (Yusuf Ahmed, 1957), there is no scientific report on the microbiology, biochemical changes and safety of this traditional product. Information pertaining to the preparation techniques of *wakalim*, microorganisms responsible for its fermentation and the microbiological safety of the final product is important for large scale production of *wakalim* using defined starter cultures. The aim of this study was, therefore, to document the traditional knowledge with respect to *wakalim* preparation processes and analyze its microbiological flora with emphasis on the fermenting lactic acid bacteria.

MATERIALS AND METHODS

Description of wakalim preparation processes

Information pertaining to the procedures of *wakalim* preparation was gathered through interview, on-site video recording and using questionnaires. The study was conducted from December, 2004 to October, 2005 in Harar, East Ethiopia. Respondents were selected randomly after preliminary screening of candidates with good knowledge of the preparation processes of *wakalim*. The respondents and traditional *wakalim* producers were approached through informants from the community. A total of 96 subjects were included in the study.

Sample collection and processing

A total of two hundred *wakalim* samples were collected from 50 different households. About four *wakalim* samples were collected per household, and analyzed for their microbiological and some physico-chemical properties. Samples were collected in sterile polythene bags and transported to the Microbiology laboratory, Science Faculty, Addis Ababa University, using cold chain. During laboratory analysis, each *wakalim* sample was surface sterilized using 70% ethyl alcohol and 25 g of its contents were homogenized in 225 ml of sterile peptone-water (0.1%) for 2 minutes using a Stomacher lab blender (Stomacher 400, Seward, UK).

Enumeration and identification

After appropriate dilution, 0.1ml aliquots were surface plated in duplicates on pre-dried surfaces of the following media (Oxoid) for microbial enumeration: Plate Count (PC) agar for aerobic mesophilic bacteria (AMB), Violet Red Bile (VRB) agar for coliforms, Violet Red Bile Glucose (VRBG) agar for members of Enterobacteriaceae and Mannitol Salt agar for *staphylococci*. The plates were incubated under aerobic conditions for 1-2 days at 30-32°C. Similarly, MRS (de-Mann, Rogosa and Sharpe) agar was plated and incubated under anaerobic condition, using anaerobic jar (BBL, GasPak Anaerobic Systems) for 2-3 days at 30-32^{0}C for counting lactic acid bacteria (LAB). Chloramphenicol Bromophenol Blue (CBB) agar was similarly plated and incubated at 25-28^{0}C for 3-4 days for yeasts and mold count (CBB consisted of: yeast extract, 6.0 g; glucose, 20.0 g; chloramphenicol, 0.1g; bromophenol blue, 0.01 g; agar, 15 g; distilled water, 1000ml; pH, 6.0-6.4). Aerobic spore counting was done on Plate Count (PC) agar after appropriate dilutions were heat-treated at 80°C for 10

minutes in water bath.

After colony counting, 10 to 15 colonies were randomly picked from countable plates of PC and MRS agar plates for further identification. Colonies of LAB were transferred into about 5ml MRS broth (Oxoid) and purified by repeated streaking on MRS agar. AMB were similarly transferred into Nutrient broth (Oxoid) for repeated purification on Nutrient agar (Oxoid). The pure cultures of LAB and AMB were streaked on slants of MRS agar and Nutrient agar, respectively, and were stored at 4^0C for further characterization.

Characterization of isolates

Isolates were microscopically characterized by cell shape, cell grouping, motility, and presence or absence of endospores. Gram reaction of isolates was tested by the KOH test (Gregerson, 1978). Production of the enzyme oxidase was tested according to Kovacs (1956) and formation of catalase was determined by flooding young colonies with 3% solution of H_2O_2. Oxidative or fermentative utilization of glucose by each isolate was assessed by the O/F test (Hugh and Leifson, 1953). The testing medium consisted of (g/l): Peptone, 2g; yeast extract, 1g; NaCl, 5g; K_2HPO_4, 0.2g; glucose, 10g; Bromophenol blue, 0.08g; agar, 2.5g; distilled water, 1000ml, pH, 7.1. Purified isolates from MRS agar plates which were Gram-positive, non-sporing cocci or rods, and which did not produce the enzyme catalase were considered as LAB and were subjected to biochemical tests using API 50CH (Biomeriuex, Marcy I'Etoile, France). The biochemical profiles of isolates were analyzed to species and subspecies level using identification software (API WEB, V1.1.0, Biomeriuex, Marcy I'Etoile, France).

Physico-chemical analysis

The pH of samples was measured using digital pH meter (Nig 333, Naina Solaris LTD, India) after homogenizing 10 g of *wakalim* in 90 ml of distilled water (Erkmen and Bozkurt, 2004). To measure titratable acidity (TA), *wakalim* sample (5g) was homogenized in 20 ml distilled water and filtered through whatman No.1 filter paper. To 20 ml of the filtrate, 2 to 3 drops of phenolphthalein were added and this was titrated against 0.05M NaOH to the end point of phenolphthalein. Titratable acidity was expressed as g lactic acid/100g of *wakalim* (Antony and Chanrda, 1997).

Data analysis

Data were analyzed using SPSS for Windows (version 10.0). Significances of differences between means of samples were computed using one-way

ANOVA. Coefficient of variation was calculated to evaluate the significances of differences among samples.

RESULTS

Description of *Wakalim* Preparation Processes

A total of 96 people gave information on raw materials used for *wakalim* making and on preparation procedures (Table 1). Most of the respondents (83%) belonged to the Harari ethnic group. The respondents consisted of merchants (25%), government employees (44%), handcraft workers (19%), and house wives (12%). All respondents were muslims. W*akalim* preparation has been the art and duty of females. The local producers of *wakalim* relied on spontaneous fermentation of raw meat and other additives. On the average, one kg of *wakalim* consisted of lean meat (700 g), fat (50 g) salt (20 g), various spices (70 g), and onion (160 g) (Table 2). Proportion of raw materials used for *wakalim* preparation varied among respondents with significant variation with regards to various spices, particularly garlic and red pepper (CV>10%).

Table 1 Socio-demographic characteristics of the study population (respondents)

Demographic characteristics	No of respondents	Proportion (%)
Sex		
Female	90	94
Male	6	6
Age		
≤30	9	9
30-40	45	47
41-50	18	19
51-60	15	16
61-70	6	6
≥70	3	3
Marital status		
Married	90	94
Single	6	6
Occupation		
Trader	24	25
Government employee	42	44
Handcraft	18	19
House wife	12	12
Ethnicity		
Harari (Adare)	80	83
Oromo	16	17
Religion		
Muslim	96	100
Christian	-	-

Table 2 Mean weight of ingredients used for *wakalim* preparation

Components*	Proportions (g/kg)	SD	CV (%)
Lean meat	700.0	50	7.14
Fat (fatty tissue)	50.0	5	10
Salt (sodium chloride)	20.0	2	10.0
Spices:			
Ethiopian cardamom (*Aframomum corrorima)*	10.0	0.7	7.0
Black cumin (*Nigella sativa*)	10.0	0.5	5.0
Kemun (*Trachyspermum capticum*)	10.0	1.0	10.0
Ethiopian mustard (*Brassica nigra*)	10.0	1.35	13.5
Garlic (*Allium sativum*)	10.0	3.5	35.0
Red pepper (*Capsicum annuum*)	20.0	6.0	30.0
Onion (*Allium asclonicum*)	160.0	20.0	11.1
Lemon (*Citrus limona*)	Juice of one average fruit (20 to 30ml/kg meat)	-	-

* In addition to the major components mentioned, very small amounts of other spices have been used to make the product spicier. These spices include clove (*Eugenia caryophylla*), cinamon (*Cinamomum zylanicum*), and cumin seeds (*Lepidium sativum*).

A series of events take place during preparation of *wakalim*. These include the preparation of casing (*Merechi*) from small intestine of an ox, course-cutting (chopping) of meat and grinding of garlic (*Allium sativum*), mixing the processed meat with other spices, and stuffing all the ingredients into the casing. Finally, the stuffed product is allowed to dry over wood smoke by suspending it on stretched wire about 2.00 to 3.00 meters above the hearth in a kitchen for 5 to 7 days. The generalized production flow chart for the production of *wakalim* is shown in Fig. 1.

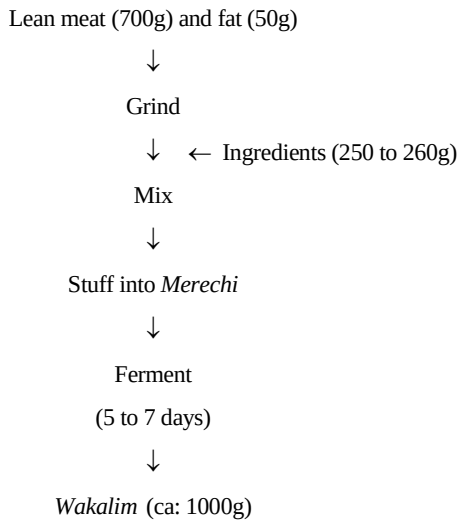

Fig. 1. Flow diagram for the production of *wakalim*

Preparation of casing (merechi)

Merechi is a casing tube made from the small intestine of an ox. From freshly slaughtered ox, about 5 to 10 m of the middle portion of the small intestine is cut off and the intestinal content removed by flushing with tap water. The flushed intestine is turned inside out and further cleaned. A vessel, large enough to accommodate the intestine portion, is half-filled with water and washing powder. The intestine is immersed in it and washed thoroughly. Finally, it is rinsed in water, and turned the right side out and re-rinsed. The intestine is tied on one end and blown by mouth until it coils itself. The other end is then tied and it is allowed to dry over direct sunlight for an hour. The ready-to-use intestine is cut in to about 20 cm length and used for stuffing.

Mixing and stuffing of ingredients

Preparation and mixing of the raw materials (ingredients) is the second major step during *wakalim* preparation. The amount of the raw materials to be mixed depends on the amount of *wakalim* required. Meat and a few heads of onion (*Allium asclonicum*) are ground (or chopped) on a cutting board in to small pieces using knife and put into a plastic vessel of about 5kg capacity. The course-cut meat and onion are mixed thoroughly by hand. Several ground spices (Table 2) are separately spread on the meat/onion mix and further mixed by hand, and to these, some ground garlic (*Allium sativum*) and salt are added. It is a common practice to squeeze one 20 to 30 gram head of lemon (*Citrus limona*) for every one kg of meat used.

Although the major ingredient used for *wakalim* preparation is lean meat, ingredients other than meat are incorporated for sensory purpose. The overall proportions of ingredients recommended for the making of *wakalim* by the local producers and consumers did not differ significantly with respect to fat, salt and spice content (CV <10%). Highly significant variations were, however, observed in cases of garlic (*Allium sativum*) and red pepper (*Capsicum annuum*) content (CV>10%).

After the washed and blown-up intestine (*merechi)* is completely dry, it is cut in to small pieces of about 20 cm long. The spiced meat is stuffed into the casing and it takes up a U-shape (Fig. 2E). Usually, the two ends of the bent *wakalim* are made to meet to form a closing.

Fig. 2. Pictorial representation of *wakalim* preparation processes. (A) Meat grinding, (B) Ground meat, onion, garlic and spices ready for mixing, (C) Meat/spice mix, (D) Casing *Merechi*, (E) Fresh stuffed *wakalim*, and (F) Fermented *wakalim*

Fermentation and drying

The stuffed *wakalim* is left to ferment for 5 to 7 days at ambient temperature. The fermented product is allowed to dry gradually by exposing it to warmer environment for some period. Usually, the local producers' dry *wakalim* by suspending it on a string (or wire) stretched a little above a traditional cooking stove (2.00 to 3.00 meters).

As fuel wood is used for cooking in traditional stoves, the product is smoked and gradually loses water ending in product of low moisture content.

W*akalim* that has passed the combined stages of fermentation and drying is ready for consumption. It is served either uncooked or fried in oil. In traditional ceremonies, it is usually fried for a few minutes before serving.

Microbiological analysis

The counts of aerobic mesophilic bacteria (AMB) and lactic acid bacteria (LAB) were over 10^7 cfu/g in *wakalim* samples (Fig. 3). ASF and *staphylococci* were among the commonly isolated microbial groups in *wakalim* samples next to AMB and LAB. Enterobacteriaceae, yeasts and molds were found at counts below detectable levels (≤ log 1 cfu/g).

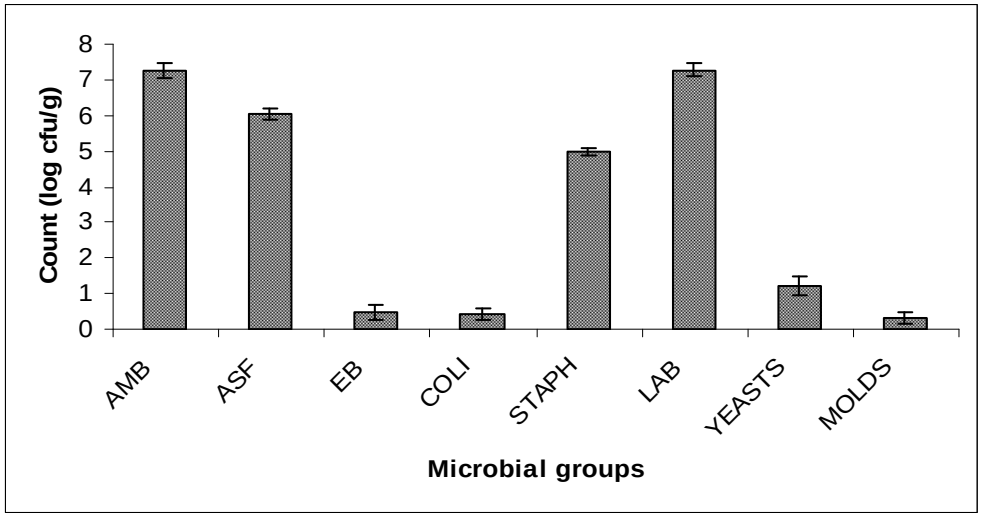

Fig. 3. Microbial profiles of some *wakalim* samples. AMB, Aerobic mesophilic bacteria; ASF, Aerobic spore former; EB, Enterobacteriaceae; COLI, Coliforms; STAPH, *staphylococci*; LAB, lactic acid bacteria.

A total of 198 LAB were isolated from fermented *wakalim* samples. Of the isolates, 90 (45%) were rod shaped while 108 (55%) were cocci.

Based on carbohydrate fermentation profiles, the LAB isolates were classified in to five genera belonging to lactobacilli (6 species), pediococci (3 species), *Leuconostoc* (2 species) and one species each of *Lactococcus* and *Weissella*. Of the *pediococci* isolates, strains of *Pediococcus pentosaceus*1 were the dominant isolates (29%) followed by *Pediococcus pentosaceus*2 (17%) (Table 3). Among the rods, *Lactobacillus plantarum*1 (19%) and *Lb. brevis*1 (16%) were the frequently isolated species. *Lb. pentosus* (4%), *Lb. brevis* 3 (3%), and *Lb. delbrueckii spp. lactis* (1%) had the lowest isolation rate among the lactobacilli. *Pediococci*, however, were the most frequent isolates (108/198).

Table 3 API 50CH fermentation profile of representative* LAB isolated from *wakalim* samples and of some reference strains

S. no	Substrate	Isolate code														
		MR406	MR415	MR423	MR482	MR500	MR156	MR1511	MR158	MR422	MR430	MR1521	MR3165	MR407	Lb.pl ATCC 8014	Lb.br ATCC 14869**
1	L-Arabinose	+	+	+	+	+	+	+	+	+	w	-	-	+	+	+
2	Ribose	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+
3	D-xylose	-	-	-	+	+	+	+	+	-	-	-	-	+	-	-
4	β-Methyl-D-Xyloside	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
5	Galactose	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+
6	D-Glucose	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
7	D-Fructose	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
8	D-Mannose	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+
9	Mannitol	+	-	+	+	-	-	-	-	-	-	-	-	+	+	+
10	Sorbitol	+	-	-	+	-	-	-	-	-	-	-	-	+	+	+
11	α-Methyl-D-Mannoside	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+
12	α-Methyl-D-Glucoside	-	-	-	-	-	-	-	-	-	w	-	-	-	+	-
13	N-Acetyl-Glucosamine	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+
14	Amygdalin	+	+	+	+	+	-	+	-	-	-	-	-	+	+	+
15	Arbutin	+	+	+	+	+	-	+	-	-	-	-	-	+	+	+
16	Esculin	+	+	+	+	+	-	+	-	-	-	-	-	+	+	+
17	Salicin	+	+	+	+	+	-	+	-	-	-	-	-	+	+	+
18	Cellobiose	+	+	+	+	+	-	+	+	-	+	-	-	+	+	+
19	Maltose	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+
20	Lactose	+	-	-	+	+	+	+	-	-	+	-	-	+	+	+
21	Melibiose	+	-	-	+	+	-	-	-	-	+	-	+	+	+	+
22	Saccharose	+	-	+	+	+	-	+	-	+	+	-	+	+	+	+
23	Trehalose	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+
24	Inulin	-	-	-	+	+	-	+	-	-	-	-	-	+	-	-
25	Melezitose	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+
26	D-Raffinose	+	-	+	+	+	-	-	-	-	-	-	-	+	+	+
27	Starch	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
28	Gentiobiose	+	+	+	+	+	-	+	+	-	-	-	-	+	+	+
29	D-Turanose	+	-	-	-	-	-	-	-	-	-	-	-	-	+	+
30	D-Tagatose	-	+	+	+	+	-	+	+	-	-	-	-	+	-	-
31	L-Fucose	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
32	Gluconate	w	-	-	-	-	+	+	-	-	-	-	w	w	+	+
% ID		99.9	99.9	99.5	88.9	99.9	90.0	99.4	84.7	99.9	91.3	99.8	99.3	95.6	99.9	99.9
No of isolates		19	29	6	4	17	3	16	1	1	1	1	1	1	1	1

Where:

MR 406 = Lactobacillus plantarum1
MR 415 = Pediococcus pentosaceus1
MR 423 = Lactococcus lactis ssp lactis
MR 482 = Lb. pentosus
MR 500 = Pediococcus pentosaceus2
MR 156 = Lb. brevis3
MR 1511 = Lb. brevis1
MR158 = Pediococcus acidolactici
MR 422 = Weissella viridescens
MR 430 = Leuconostoc lactis1
MR 1521 = Leu. mesenteroides spp. mesenteroides
MR 3165 = Lactobacillus delbrueckii spp lactis
MR 407 = Lactobacillus brevis
Lb. pl ATCC 8014 = reference strain, Lb. plantarum
Lb. br ATCC14869= reference strain, Lb. brevis

* More than one isolates of the same species were represented with one of the species with identical or better percent identification. +, = assimilated (fermented); -, not assimilated (not fermented); w, doubtful or weak;
**Assigned to *Lb. plantarum1* by API 50CH

Wakalim samples had mean pH of 5.35 ± 0.8 and mean titratable acidity (TA) of 6.38% ± 0.69% (Table 4). There was, however, significant variation among *Wakalim* samples in pH and TA values (CV>10%).

Table 4 pH and titratable acidity of some w*akalim* samples

Parameters	Sample size	Mean ± S.D	Minimum	Maximum	CV (%)
pH	200	5.3465 ± 0.8407	4.13	6. 28	15.72
TA	200	0.0638 ± 0.0069	0.02	0.079	10.82

TA, titratable acidity; S.D, standard deviation; CV, coefficient of variation

DISCUSSION

The procedure for traditional preparation of *wakalim* has certain similarities particularly to that of European-style dry fermented sausages. Both involve mixing or blending of ingredients, stuffing into casing made of animal intestine, and fermentation. Moreover, reliance on spontaneous fermentation without the use of starter cultures, the use of natural casing, casing diameter, utilization of low fat, and fermentation period are among the similarities shared between *wakalim* and French traditional dry fermented sausage. However, French traditional fermented dry sausages making additionally requires the use of sugar, higher salt concentration, and lower fermentation temperature (Lebert *et al.* 2006). The production processes of *wakalim* also share certain features with that of raw fermented sausages made in Germany (Bremer *et al*., 2004) in which curing, smoking, fermentation and drying are crucial steps. Although sodium chloride (NaCl) has been used at a proportion of 2%, neither nitrate nor nitrite has been used in the making of *wakalim.* The European-style fermented sausages are, in general, more diverse than *wakalim* as they are made from various combinations of ingredients (different recipes), using casings of different animals (sheep, hog, cattle, etc) with varying diameters (2 to 10 cm) (Flores, 1997). The casing used for *wakalim* making is of bovine origin with an average diameter of about 3cm.

Wakalim is one of the traditional foods highly valued among the Harari people. It is not an every-day food at household level, but is prepared and served on notable occasions, such as wedding parties, '*Ramadan*' feast, 'Arafat' feast, and during pilgrimage. It is prepared in large quantities at wedding parties. At every meal to which the brides are invited, serving *wakalim* among the variety of dishes is a rule than an exception (Yusuf Ahmed, 1957). The preparation of *wakalim* is not only a method of food

preservation, but also a source of income. In addition, preparation and consumption of *wakalim* is an expression of the cultural identity of the Harari community. Currently many of the cafés, some restaurants and Women's Cooperative Associations in Jegol and Harar town sell *wakalim* as ready-to-eat food on regular basis.

Although produced and consumed typically by Hararis, other Muslim communities residing in and around Harar also prepare and consume *wakalim*. Currently, more than 30 small enterprises (cafés, restaurants, and Women's Cooperative Associations) are involved in the selling of *wakalim*. The town of Harar has a population of 77,000 and serves as one of the commercial, religious, and political centers in Eastern Ethiopia and is home to various ethnic groups (Solomon Worku and Mesganaw Fantahun, 2006). According to the 1994 census, the Harari people living inside Jegol (the walled city) were estimated to be 30, 000 (ECSA, 1995).

Although wakalim is produced following the same traditional technique, there could be variation among households and vending houses with regard to the weight (200 to 250grams), quality and prices (5 to 10 Ethiopian Birr) of wakalim. With an average sales rate of about 20 to 25 *wakalim* per day per vending house, the vendors sustain the supply of *wakalim* to the market through regular production of 40 to 50 *wakalim* per day/vending house besides its bulk production on special orders. Thus, wakalim production has both cultural and economic significance.

Both producers and consumers of *wakalim* are aware of the fact that the product has good keeping quality with shelf-life of more than 3 to 5 months. Thus, *wakalim* has been the food of choice when they are away from home for longer periods (e.g., pilgrim, war periods). The tradition of *wakalim* preparation is a good example of preservation of excess meat through minimizing food loss due to microbial spoilage. According to local producers and consumers of the product, the keeping quality is highly dependent on physical factors including temperature and smoking. Due attention has not been given to the role of microorganisms in the development of flavor, aroma and wholesomeness of the product.

The different ingredients used for *wakalim* preparation could play an important role in improving flavor and safety of the final product. Salt (NaCl, 2%) acts as one of the hurdles against the growth of some undesirable microorganisms. It also induces the solubilization and diffusion of myofibrillar proteins from muscle to form a gel texture between meat and meat as well as meat and fat particles of the raw sausage material (Lucke,

1985). Spices, such as pepper, cardamom and garlic have an impact on flavor and they may also have anti-oxidative and antimicrobial effects (Hammes, 1977).

Wakalim samples contained different groups of microorganisms. AMB, LAB, *Bacillus* spores, and *staphylococci* were encountered in *wakalim* samples in counts between log 5 cfu/g and log 8 cfu/g. As different spices have been used as ingredients during *wakalim* preparation, the dominance of *Bacillus* species was expected. A recent study made to evaluate the prevalence of ASF in some Ethiopian spices revealed high counts of *Bacillus* species (Feleke Moges and Mogessie Ashenafi, 2000). The count of *Bacillus* species may be the count of their spores as most of *Bacillus* species do not thrive well at pH values observed in most *wakalim* samples. In agreement with our observation, different groups of LAB, and *staphylococci* were reported from various traditional fermented sausages (Rantsiou *et al*., 2005). *Lactobacillus sakei*, *Lb. curvatus*, and *Lb. plantarum* were most frequently isolated from acid-fermented meat products, while different biotypes of *Staphylococcus xylosus* were the dominant *staphylococci* in traditional Italian fermented sausages (Aymerich *et al*., 2003). In our study, the dominant LAB isolated from *wakalim* samples included *Pediococcus pentosaceus*1, *Ped. pentosaceus*2, *Lb. plantarum*1, *Lb. brevis*1, *Lb. pentosus* and some unidentified species of the genera *Pediococcus* and *Lactobacillus*.

Counts of AMB, ASF, EB, *staphylococci*, LAB, yeasts and molds did not show significant variation among samples (CV< 10%). However, the counts of some groups were higher (log 5 cfu/g) and those of others were lower (log 2 cfu/g). The observed differences in microbial counts could be accounted to differences in the microbial load of the ingredients, and the hygienic quality of the raw meat used for fermentation. Differences in pH and counts might also be due to the duration of fermentation. Premature vending would result in some products with higher pH (6.28) that would permit the survival of some members of Enterobacteriaceae, albeit, at very low levels (≤ log1 cfu/g). The fact that Enterobacteriaceae and coliforms were below detectable levels in most samples (<1 log cfu/g) could possibly be attributed to the antimicrobial activity of some of the spices in combination with low pH of the samples. The ambient temperature at which the sausages were made to ferment could also affect the microbial load and composition. Although many of the local producers suspended the fermenting *wakalim* at heights of about 2.00 to 3.00 m above the source of an open fire in a traditional kitchen, some used reduced height. Under the

latter condition, the fermenting sausages could have an ambient temperature as high as 50^0C. Such an arrangement could stress the microbial communities in the fermenting *wakalim*, thus accounting to the variability in counts of the microbial groups.

Strains of LAB isolated from *wakalim* samples had certain similarities and differences to the compositions of LAB reported earlier from other traditional dry fermented sausages. Accordingly, the main agents responsible for the indigenous fermentation of traditional Italian sausages were *Lb. sakei*, *Lb. plantarum, Lb. curvatus,* and *Lb. farciminis* (Rebecchi *et al*., 1998). Similarly, two of the traditional Italian fermented sausages (salsiccia and soppressata) produced in artisanal and industrial plants were dominated by *Lactobacillus sakei* (50%) and *Pediococcus species* mainly *Ped. pentosaceus* (22%) with low prevalence of species of *Leuconostoc, Lb. plantarum,* and *Lb. curvatus (*Parente *et al.,* 2001). *Lb. sakei* dominated many of the spontaneously fermented dry sausages produced in Southern Europe (Samelis *et al*., 1998). Although phenotypic characterization failed to show presence of *Lb. sakei* in *wakalim* samples, characterization of LAB using 16S rDNA (data not given) showed the possible presence of *Lb. sakei* in *wakalim.* As could be observed from the data presented, slight difference in species composition is seen between our findings and the findings of other workers (Samelis *et al*., 1998). These differences might have arisen from differences in initial contamination level, fermentation temperature and/or the methods of characterization. In sausage fermentations below 25^0C (at 18 to 23°C), the indigenous microflora were mostly dominated by strains of *Lb. sakei* and *Lb. curvatus* (Lucke, 1998); *Lb. plantarum* predominated at higher ripening temperature (Lucke, 1985).

The counts of both yeasts and molds were below detectable levels (<log 1 cfu/g) in all *wakalim* samples. Reports on the significances of yeasts on aroma formation and sensory quality of dry-fermented sausages are controversial (Flores *et al*., 2004). Absence of yeasts in our *wakalim* samples could minimally affect the product's sensory quality. The proteolytic and lipolytic activities by some of the LAB and meat endogenous enzyme could compensate for absence of yeasts (Fadda *et al*., 1999).

Our study showed that *wakalim* fermentation would normally result in products with low pH (4.3). The higher pH in some samples could be attributed to selling of either under-fermented *wakalim* or *wakalim* on incipient spoilage. In the latter case, proteolytic activities by microbes could

result in rise of the final pH. The low pH was among the hurdles that inhibited growth and survival of members of the family Entrobacteriaceae including coliforms. Most of the fermented *wakalim* samples analyzed for the presence of coliforms and other members of the family Enterobacteriaceae had counts below detectable levels. If and when present, the counts did not exceed 3 log cfu/g.

There was a significant variation in pH and titratable acidity (CV>10%) among the samples. This variation could be due to differences in the strain of fermenting LAB or due to stage of fermentation reached when the samples were analyzed. Based on the mean pH of samples analyzed, *wakalim* as prepared at household level, could be categorized under low acid fermented meat product although the pH of some of the samples was as low as 4.13. According to Aymerich *et al*. (2003), low acid fermented meat products are those products with final pH of 5.3 to 6.2. With respect to its low moisture content, *wakalim* could be considered as dry fermented sausage (Jay, 1996).

Wakalim is served either fried or without any heat treatment. Although members of Enterobacteriaceae are below detectable levels in non heat-treated *wakalim*, *staphylococci* remained at levels around log 5 cfu/g. In the presence of high background flora and with *Staphylococcus* count of < log 7 cfu/g, enterotoxin production may not be eminent (Jay, 1996). However, considering the possible time/temperature abuse during fermentation, heat-treatment of *wakalim* before consumption could be considered a reasonable safety measure. The association of food-borne outbreak and consumption of non-heat treated fermented meat products has been documented (Adams and Moss, 1995). Following an outbreak of salmonellosis in the UK associated with a salami stick product imported from Germany, the production process was changed to incorporate a final pasteurization step (Adams and Moss, 1995).

To conclude, this study was the first of its kind on the microbiology of *wakalim,* a traditional Ethiopian fermented beef sausage. *Wakalim* belongs to dry fermented traditional sausage with high acidity and low moisture content. The acidity, low moisture content and the presence of various spices could contribute to the low count of spoilage and pathogenic microorganisms. A variety of LAB might be involved in spontaneous fermentation of *wakalim,* where *Lb. plantarum* and *Ped. pentosaceus* were the dominant strains.

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