
EVALUATION OF THE SPOILAGE POTENTIAL OF SELECTED BACTERIAL ISOLATES ON ETHIOPIAN SAUCES AND EFFECT OF TWO MAJOR SPICE FORMULATIONS ON SPOILAGE MICROORGANISMS

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ABSTRACT: The spoilage potential of *Bacillus* spp., *Micrococcus* spp., Enterobacteriaceae, *Aeromonas* spp. and other Gram positive rods isolated from spoiled traditional Ethiopian sauces was tested on legume-based, vegetable-based and meat-based Ethiopian sauces. Legume-based sauces were spoiled by all 56 test organisms within 48 h at ambient temperatures. Foul odour with or without gas production was detected at spoilage. Meat-based sauces and vegetable-based sauces were spoiled only by 14 and 12 test strains, respectively. Enterobacteriaceae were the major spoilers of most sauces in terms of foul odour and gas production. One of the major ingredients of Ethiopian sauces, the hot spice "berbere", showed stronger inhibitory property against spoilage microorganisms until 12 hours of storage.

Key words/phrases: Antibacterial effect, pepper, sauces, spoilage potential, turmeric

INTRODUCTION

Ethiopian sauces are usually hot sauces made of a variety of ingredients. Different sauces have different flavours depending on the type and amount of spices and other constituents, the extent of cooking and other factors. The sauces are basically legume-based, vegetable-based or meat-based. Legume-based sauces are usually frequented in low-income families and during fasting periods, whereas meat-based sauces are mostly luxuries for most families. In

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most households, sauces are usually prepared early in the day and are supposed to last until dinner and frequently are kept overnight at ambient temperatures.

Spoilage of sauces is detected after 24 h and is manifested in the form of foul odour and/or gas production. As the cooking temperature is over 85° C, most microorganisms in raw materials are expected to be eliminated. Spoilage may, thus, be caused by spore formers or heat resistant types. Spoilage by heat sensitive species may be due to introduction of these microorganisms after cooking. The ingredients of the various spices may also affect the extent of proliferation of spoilage microorganisms in the sauces.

The presence of spoilage and pathogenic microorganisms in prepared foods was reported by various workers (Bryan *et al.*, 1992; Ferrer *et al.*, 1992; Khan *et al.*, 1992). There is, however, very little information on spoilage of Ethiopian foods (Mogessie Ashenafi *et al.*, 1995). The purpose of this study was to evaluate the spoilage potential of some bacterial strains isolated from spoiled sauces and to assess the effect of the major ingredients of sauce spices on the spoilage microflora.

MATERIALS AND METHODS

Test strains

Fifty six isolates which constituted the dominant spoilage flora of legume-based, vegetable-based, and meat-based sauces in a previous study (Mogessie Ashenafi, 1996) were used to evaluate their spoilage potential. The isolates consisted of *Bacillus* spp. (18), *Micrococcus* spp. (6), Enterobacteriaceae (16), *Aeromonas* spp. (3), *Pseudomonas* spp. (4) and other non-spore forming Gram positive rods (9).

Preparation of sauces

Legume-based, vegetable-based and meat-based sauces were prepared following traditional methods and the sauces were sterilized in screw cap bottles in 200 ml amounts.

Inoculation with test organisms

Test strains were grown overnight in Brain Heart Infusion Broth (Merck), centrifuged and washed repeatedly in sterilized water and were separately inoculated into the various sauces to give an inoculum level of 10^2 - 10^3 cfu(ml)⁻¹.

Inoculated sauces were kept at ambient temperature and checked periodically at 12 h intervals for signs of spoilage (foul odour or gas production).

Effect of major sauce spices on spoilage microflora

The effect of the two major spices, "berbere" and "erd", used to prepare hot or mild sauces, respectively, on spoilage microflora was studied. The traditionally prepared powdered hot spice "berbere" constituted of red mature fruits of pepper (*Capsicum annum*) (70%), *Nigella sativa* (3%), *Trachyspermum ammi* (3%), Thyme (3%), false cardamom (7%), Ginger (2%), rue fruits (2%), garlic (3%), fenugreek (0.1%) and salt (7%). Most households prepare their own "berbere", although, in urban areas, it can be purchased from vendors.

Based on the proportion used in traditional sauce making, "berbere" medium was prepared at 10% concentration in Brain Heart Infusion Broth. The mild spice "erd" consisted of turmeric and was prepared by making a 0.2% mixture of turmeric in Brain Heart Infusion broth.

Eight isolates which produced strong foul odour and/or gas in the spoilage experiment were separately inoculated in "berbere" or "erd" medium at an initial inoculum level of 10^2 or 10^3 cfu(ml)⁻¹. Brain Heart Infusion broth without "erd" or "berbere" served as control.

Samples were taken every two hours and plated on Brain Heart Infusion Agar to determine their growth rates.

RESULTS

The test strains had various spoilage potential in the different sauce types (Table 1). All test strains spoiled legume-based sauce within 48 h at ambient temperatures. Of these, 14 produced gas in addition to foul odour and they consisted of Enterobacteriaceae (10), *Bacillus* spp. (3) and *Aeromonas* spp. (1). These strains were originally isolated from spoiled legume-, vegetable, or meat-based sauces.

Only 14 strains were able to spoil meat-based sauces. Eight exhibited spoilage activity already within 48 h, and the remaining at 70 h. Foul odour and strong gas was produced by *Aeromonas* (1), Enterobacteriaceae (11) and *Bacillus* spp. (1).

Table 1. Spoilage pattern of the various sauces by test organisms.

Test organism	Number tested	Legume-based		Vegetable-based		Meat-based	
		Foul odour	Gas	Foul odour	Gas	Foul odour	Gas
<i>Bacillus</i> spp.	18	16	3	1	-	2	1
Micrococci	6	6	-	-	-	-	-
Other G ⁺ rods	9	9	-	-	-	-	-
Enterobacteriaceae	16	15	10	10	10	11	11
<i>Aeromonas</i> spp.	3	3	1	1	1	1	1
<i>Pseudomonas</i> spp.	4	4	-	-	-	-	-

Vegetable-based sauces were spoiled only by 12 strains, but spoilage was detected as early as 42 h in all cases. Foul odour and gas production were frequently observed in sauces inoculated with Enterobacteriaceae.

Eight Enterobacteriaceae and one *Aeromonas* isolates resulted in spoilage of sauces of all types.

Of the six Enterobacteriaceae test strains, four showed no significant difference in rate of growth in control or "erd" medium ($p < 0.1$). Significantly lower rate of growth was seen in "berbere" medium than in control in four strains ($p > 0.05$). "Berbere" medium also showed a significantly retarding effect than "erd" medium on four strains ($p < 0.025$). The single test strain of *Aeromonas* showed significantly retarded growth in "berbere" medium than in control ($p < 0.025$). No significant difference was observed in growth of *Bacillus* test strains in control and "erd" medium. Growth rate was, however, significantly lower in "berbere" medium than in control ($p < 0.025$) or in "erd" medium ($p < 0.005$). In general, "berbere" showed a stronger growth retarding effect on the test strains than turmeric (Figs 1 and 2). Final counts at 24 h were, however, not different in all media including the control.

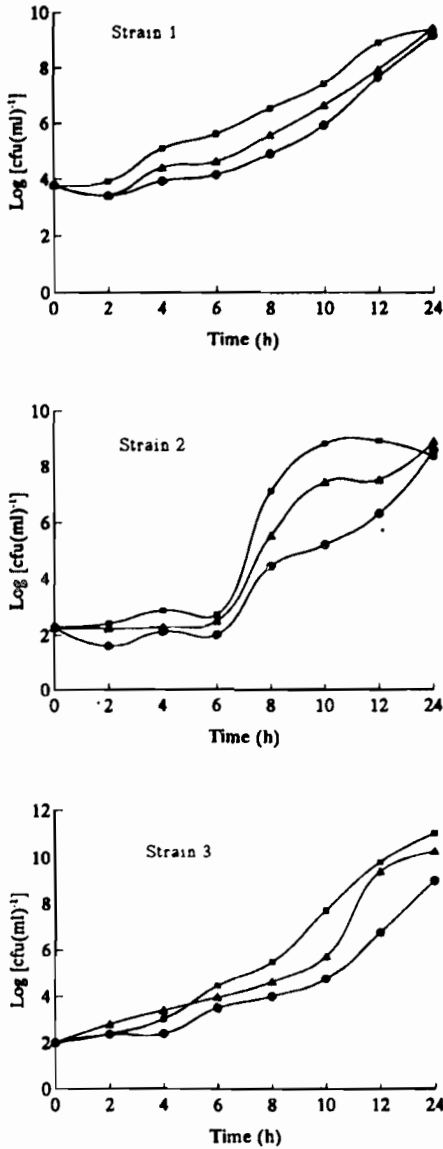


Fig 1. Growth pattern of three Enterobacteriaceae test strains in control (■), turmeric medium (▲) and “berbere” medium (●).

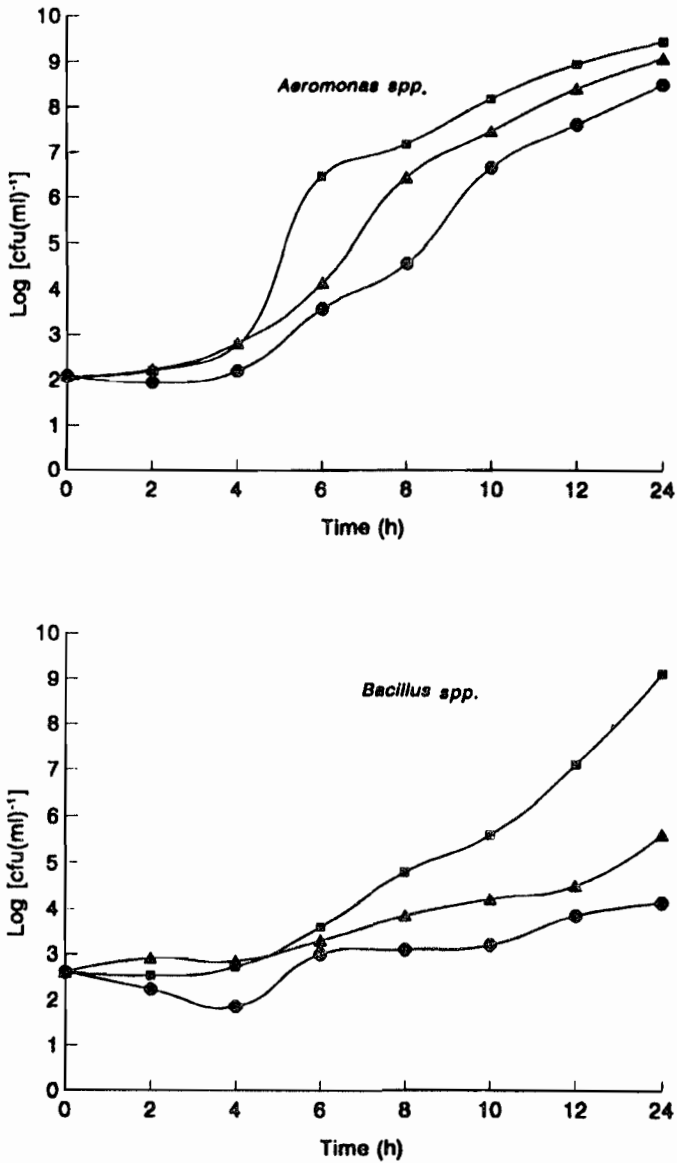


Fig 2. Growth pattern of *Aeromonas* spp. and *Bacillus* spp. test strains in control (■), turmeric medium (▲) and "berbere" medium (●).

DISCUSSION

Spoilage of the various traditional sauces resulted in foul odour and was of effervescent or non-effervescent type. Normally a family rejects any sauce which manifests any one of the two spoilage types. Effervescent type of spoilage was more common in legume-based sauces and the major gas producer in such sauces were Enterobacteriaceae.

Meat and vegetable-based sauces were spoiled only by a limited group of microorganisms. Enterobacteriaceae were the major spoilers of these sauces and spoilage was mainly of foul odour and effervescent type. The time/temperature combination during the cooking of all types of sauces is sufficient to kill Enterobacteriaceae, which, in this study, were found to be the major spoilers. It is, thus, evident that Enterobacteriaceae, which were introduced after cooking or during serving should be the major causes of spoilage. Microorganisms can be introduced into the sauces from serving spoons. As left-over sauces are not usually reheated and are kept at ambient temperatures until the next serving, introduced microorganisms may proliferate in the sauces and cause spoilage.

Bacillus and *Aeromonas* test strains also spoiled the three types of sauces. Only very few *Bacillus* test strains produced gas and this was in agreement with the observations of Al-Diejaili and Anderson (1991), where they caused non-effervescent spoilage in under processed foods. The *Aeromonas* test strains produced foul odour and gas. *Aeromonas* spp. were reported to contaminate a variety of food types (Barnhart and Pancorbo, 1992; Krovacek *et al.*, 1992).

Vegetable-based sauces and meat-based sauces were relatively more resistant to spoilage by a variety of spoilage microorganisms. Although various vegetables are initially contaminated with a variety of microorganisms (Khan *et al.*, 1992), the persistence of vegetable-based sauces to spoilage may be due to the occurrence of antimicrobial activities in their juices as observed by Marchetti *et al.* (1992). Meat-based sauces are usually cooked much longer than other sauce types and are strongly spiced. In addition to the effect of thorough cooking, spices may exhibit some inhibitory effect on certain microorganisms (Aureli *et al.*, 1992).

One of the major ingredients of Ethiopian hot sauces is "berbere". This ingredient has shown a stronger, but not long lasting, inhibitory property

against the spoilage microorganisms. As "berbere" consists of a variety of different spices, its retarding effect may be due to the antimicrobial effect of some essential oils from the spices as observed by Aureli *et al.* (1992). However, as retardation was effective only for the first 12 hours, this ingredient may not be expected to improve the keeping quality of sauces stored overnight.

It may be difficult to use cold storage in most households in Ethiopia. However, reheating sauces satisfactorily after servings may help to improve the keeping quality of the sauces even when stored at ambient temperatures.

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