

ANTIBACTERIAL ACTIVITY OF SOY SAUCE AS A FERMENTED SEASONING PRODUCT

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(Manuscript received 27 July 2004)

Abstract

Soy sauce is a popular fermented product in world wide used in the preparation of salads , soups as well as a seasoning product. It contains some compounds which may delay microbial growth. The behavior of five strains of pathogens (*Escherichia coli* , *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*) were studied in the fermented soy sauce at both 4°C and 30°C to detect its antibacterial activity. Numbers of *E.coli* and *Staph. aureus* showed a decrease , to undetectable level (<30 cfu/ml) within 48 hrs in soy sauce at 30 °C, while at 4°C their numbers exhibited nearly a constant values throughout the same period. *Pseudomonas aeruginosa* cells scored a limited growth behavior than the previous strains when stored at 30 °C for 9 hrs while the cells completely died off at 24 and 48 hours. Numbers of the same strain demonstrated a constant values when stored at refrigeration. Cells of *Salmonella typhimurium* as well as *Bacillus cereus* showed a slight decrease in their numbers then complete die off after 6 days of incubation at 30°C. At low temperature (4°C) the latter strains could grow in considerable numbers. Soy sauce exhibited an antibacterial activity against the growth of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. The diameter of inhibition zone of the tested organisms were 21,19,14,25 and 13 mm, respectively. Generally, soy sauce contains an antibacterial activity against pathogens and spoilage organisms when stored at room temperature while , at refrigeration this activity is less announced. It is believed that antimicrobial activity of soy sauce is due to soy sauce components produced from the fermentation process and the concentration of Nacl.

INTRODUCTION

Soy sauce is the most popular flavoring product used world wide and especially in the oriental countries. In general, soy sauce is a dark – brown liquid extracted from a fermented mixture of soybean and wheat and known as meat like flavor. It has a salty taste, and sharp flavor. Soy sauce has been served as all purposed seasoning and as a flavoring agent for many foods. In preparation of soy sauce two fermentation stages occur, in the first one, solid state occurs during the so – called koji making, where various enzymes are produced under aerobic conditions. In the second stage (known as brine fermentation) the process occurs under anaerobic conditions. In this stage,

enzymes from koji hydrolyze proteins to yield peptides and free amino acids . Starch also is converted to simple sugars. Lipids, on the other hand are hydrolyzed by lipases into fatty acids as reported by (Ensminger *et al.*, 1996). In recent years some fermented soy foods have gained popularity in Egypt and are being commercially produced because of its lower cost than other traditional food products. Recent nutritional and medical research have revealed the strong potential of soy foods for lowering blood cholesterol levels and the incidence of heart disease.

(Kinoshita *et al.*, 1997) identified new isoflavone in soy sauce during manufacturing by fermentation. Many desirable biological activities, uch as antifungal, antioxidative and estrogenic activities, were found in various flavonoids.

No cells of *Clostridium botulinum* could grow in either types of soy sauce contained 9 % or 17% NaCl for 3 months at 30°C throughout the storage period. The bactericidal activity of fermented soy sauce against nine kinds of intestinal pathogenic bacteria including *Escherichia communis* , *Shegilla flexneri*, *Vibrio cholera* , *Salmonella typhi*, and *B . subtilis* were confirmed . The bactericidal activity in soy sauce was attributed to the acidity, high osmotic pressure, and some chemical components (Ujii and Yokoyama, 1956). (Wang *et al.*, 1972) found an antimicrobial activity for molds commonly used in oriental food fermentation. This activity was determined by paper disc assay with *Bacillus subtilis* as a test organism. (Masuda *et al.*, 1998) reduced the cell numbers of bacteria at 18 or 4°C to a lesser extent than at 30°C in soy sauce.

(Yokoigawa *et al.*, 1999) found that 12.5% soy sauce allowed the growth of *E. coli* °157:H7 strains and while reduced the viable cell numbers of nonpathogenic *E.coli* strains. (Calicioglu *et al.*, 2002a) Studied the inactivation of acid - adapted and non-acid - adapted *Escherichia coli* 0157:H in beef jerky (treated with 86 % soy sauce) during drying process. (Calicioglu *et al.*, 2002b) reported destruction of acid and non acid - adapted *Listeria monocytogenes* inoculated in beef jerky (treated with 86 % soy sauce) during drying process. (Calicioglu *et al.*, 2003 a,b) found that marinated jerky(treated with 86 % soy sauce) during process and the low water activity of the dried product provide antimicrobial effects against possible post processing contamination with (*Listeria monocytogenes*, *E. coli* °157:H and *Salmonella*) .

The aim of this investigation is to study the antibacterial activity of soy sauce against some pathogens.

MATERIALS AND METHODS

1- Microorganisms

a) Pathogenic bacteria

Five strains of bacteria from the belonging of the American Type Culture Collection (ATCC) were obtained from the Microbiology Department ,Faculty of Agriculture, Cairo University. These strains were *Escherichia coli*, ATCC 25922, *Staphylococcus aureus*, ATCC 25923 , *Bacillus cereus*, ATCC 33018, *Pseudomonas aeruginosa*, ATCC 9027, and *Salmonella typhimurium*, ATCC 20231. Culture were maintained on nutrient agar slant at 4 °C and sub - cultured in nutrient broth for 24 hours at 37 °C prior to inoculation.

b) Fungi

A strain of *Aspergillus oryzae* used in the production of soy sauce was obtained from the laboratory of Food Science and Technology Institute, Korea by Personal communication .

2- Media

Malt agar slants is the medium used for the growth and maintainance of pure cultures (APHA , 1976). Nutrient broth was used for the activation of the tested organism before inoculation (APHA, 1980). Tryptic glucose agar was used for the enumeration of test organism through the experiment (AOAC , 1980).

3 - Antimicrobial activity of soy sauce

a) Inhibition Zone

Paper – Disc plate method was used to study the effect of soy sauce on bacterial growth by measuring the inhibition zone (Loo *et al.*, 1945). Two petri dishes were filled with 15 ml tryptic glucose agar medium inoculated with 0.5 ml of tested organism (1×10^5 cfu/ml). After the agar had solidified two sterilized filter paper whatman No 1 (disc) were immersed in soy sauce for 3 seconds , then were placed on the agar surface . A third petri dish was only inoculated with the tested organism as a control. The petri dishes were kept in a refrigerator for 2 hrs. for diffusion. Plates were then incubated at 37 °C for 24-48 hours and the inhibition zone was accurately measured (mm) .

b) Growth of pathogens in soy sauce

The behavior of some pathogenic bacteria, e. g., *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*, were individually studied in ordinary soy sauce (soybeans and wheat). The behavior of each

tested organisms inoculated in soy sauce was followed at both refrigeration (4 °C) and / or room (30 °C) temperatures. Counts of the tested organism were followed at intervals of initially 0,3,6,9 hours and after 1,2,3,4, and 5 days according to the procedure mentioned in of APHA. (1978).

RESULTS AND DISCUSSION

The growth of some pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium* was followed in soy sauce incubated at both 4 and 30°C. Fig. (1) shows the behavior of *E. coli* cells in soy sauce at 4 and 30°C. From the results, numbers of *Escherichia coli* in soy sauce kept at 30 °C decreased markedly and reached a minimum of 2.1×10^2 cfu/ml after 24 hours then showed undetectable level at the end of the storage period (48 hours). At 4°C *Escherichia coli* cells showed a slight decrease after 6 hours then remained constant. It could be concluded that growth of *E. coli* in soy sauce would be controlled under room temperature (30 °C). The viable counts remained constant when stored at low temperature even after 48 hours of incubation.

Data in Figures (2,3) reveal the survival patterns of *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells in soy sauce at both 4 or 30°C. Cells of *Pseudomonas species* completely died off after 24 hrs of incubation at 30 °C while, cells remain still active at 4°C at the same incubation time . Cells of *Staphylococcus aureus* found more resistant than *Pseudomonas aeruginosa* in soy sauce at both 30 °C and 4 °C, where cells of the former disappeared after 48 hrs while the second organism disappeared after 24 hrs of incubation at 30 °C. From the results obtained, it could be concluded that the elimination of *Staphylococcus aureus* and *Pseudomonas aeruginosa* populations in soy sauce was faster at room temperature than at refrigeration (4 °C).

Data presented in Fig. (4) show the growth curve of *Bacillus cereus* in soy sauce at either 30 or 4 °C. The results indicated that, *Bacillus cereus* cells in soy sauce at 30 °C showed slightly lower survival counts until 48 hrs, then dropped to undetectable limit (1.2×10^2 cfu/ml) after 5 days of incubation, while at 4 °C, the *Bacillus cereus* cells remained unchanged in soy sauce at (10^5 cfu/ml). As mentioned with *E. coli*, *B. cereus* cells also showed more pronounced antibacterial activity in soy sauce at 30 °C than at 4 °C. *B. cereus* cells have found more resistant in soy sauce than cells of *E. coli*.

The effect of storage temperatures on the behavior of *Salmonella typhimurium* cells in soy sauce is presented in Fig. (5). Viable counts of *S. typhimurium* decreased reaching to its lowest level (1.6×10^2 cfu/ml) at 30°C after 5 days of incubation. While, at 4°C survival numbers of the tested organism remained constant during the whole period of experiment. As a conclusion, no change was observed in counts *S. typhimurium* in soy sauce kept at 4°C, while at room temperature cells showed some inhibition.

Table (1) show the inhibition zone of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium* as affected by the soy sauce (fermented product). The diameters of inhibition zone of the tested organisms were 21,19,14,25 and 13 mm, respectively. Concerning the previous data, soy sauce had an antibacterial activity against all the pathogenic strains used in this investigation. Results from the table also show that *Pseudomonas aeruginosa* had the highest inhibition zone followed by *E.coli* while, *Salmonella typhimurium* and *B. cereus* revealed more resistant. These data agree also with the previous findings on the growth patterns of these organisms in commercial soy sauce.

Many investigators have mentioned that antibacterial activity in soy sauce is produced by molds commonly used in oriental fermented food Van Dijck and Desomer, 1958, Wang *et al.*, (1969) and Somkuti and Walter (1970). Results in this investigation agree with the findings of (Wang, *et al.*, 1972) who found that *Aspergillus spp.* and *Rhizopus spp* (commonly used in oriental food preparation) had antimicrobial activity against both Gram - positive and Gram - negative bacteria when occurred in oriental foods. They Also assured this fact and determined the antimicrobial activity by paper disc assay.

Data in this study agree with that obtained by (Hurst and Hughes 1983) who reported that *Staphylococcus aureus* counts (present in chicken meat slurry supplemented with soy sauce) and kept at 37 °C had been died off.

Results related to the behavior of *E. coli* in soy sauce agree with the findings of (Masuda *et al.*, 1998) who reported that the cell numbers of *E.coli* O157:H7 decreased to undetectable level (<30 cfu/ml) within 9 days of incubation in soy sauce at 30°C. While (Yokoigawa *et al.*, 1999) proved that incubation of *E.coli* strains (O157:H7 and non – pathogenic) in the presence of 12.5% soy sauce allowed the growth of *E.coli*(O157:H7) but reduced the viable cell numbers of non – pathogenic *E. coli* strain reduced. In addition (Calicioglu *et al.*, 2002a) found, inactivation of *E. coli* (Acid

adapted and non adapted strain) during drying and storage of beef jerky treated with soy sauce as a marinated component.

Results in this investigation related to the behavior of *Salmonella typhimurium* agree with the observation of (Calicioglu *et al.* 2003b) who found antibacterial effects during marinating of beef jerky with soy sauce against possible post processing contamination with *Salmonella spp.* during storage time (60 days).

Fig 1. Growth curve of *Escherichia coli* in soy sauce at both room and refrigeration temperatures.

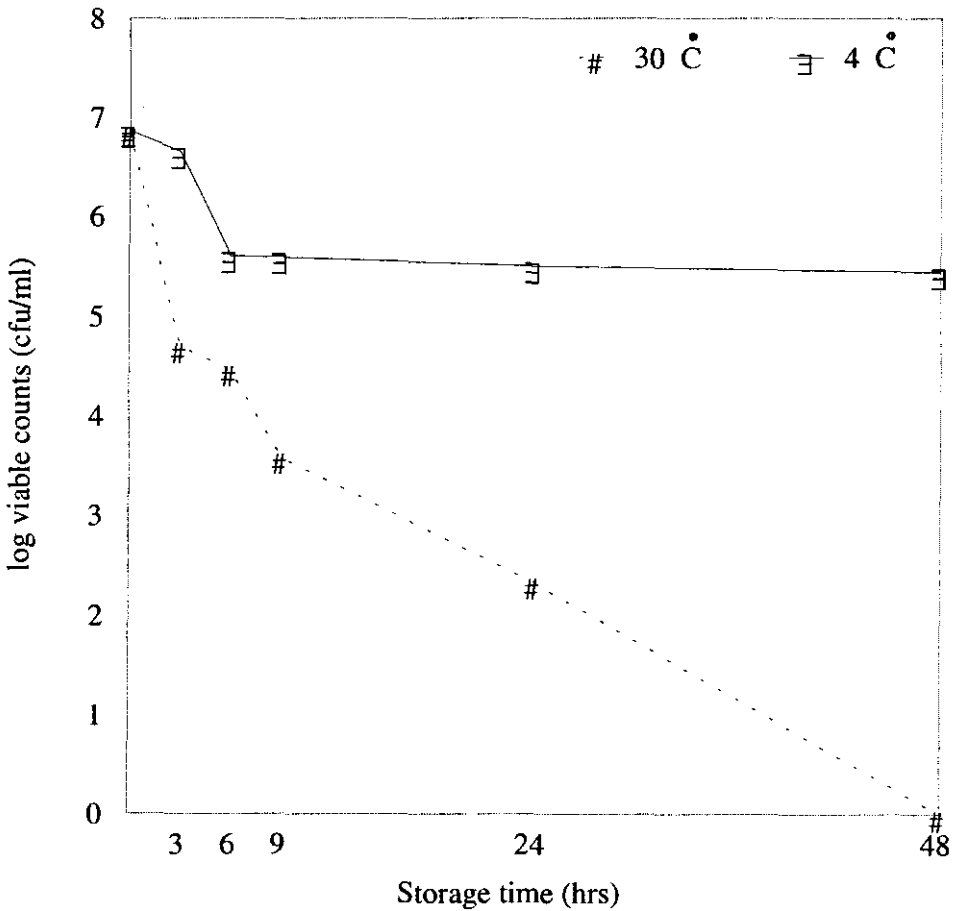


Fig 2. Growth pattern of *Pseudomonas aeruginosa* in soy sauce at both room and refrigeration temperatures.

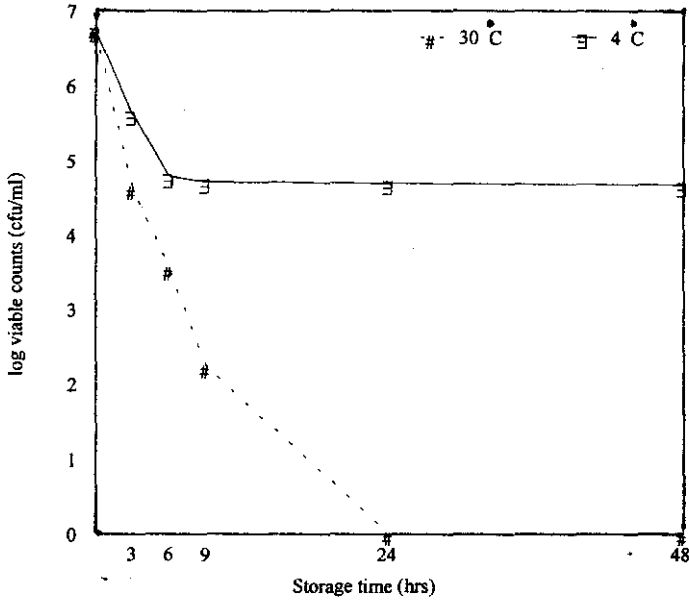


Fig 3. Growth behavior of *Staphylococcus aureus* in soy sauce at both room and refrigeration temperatures.

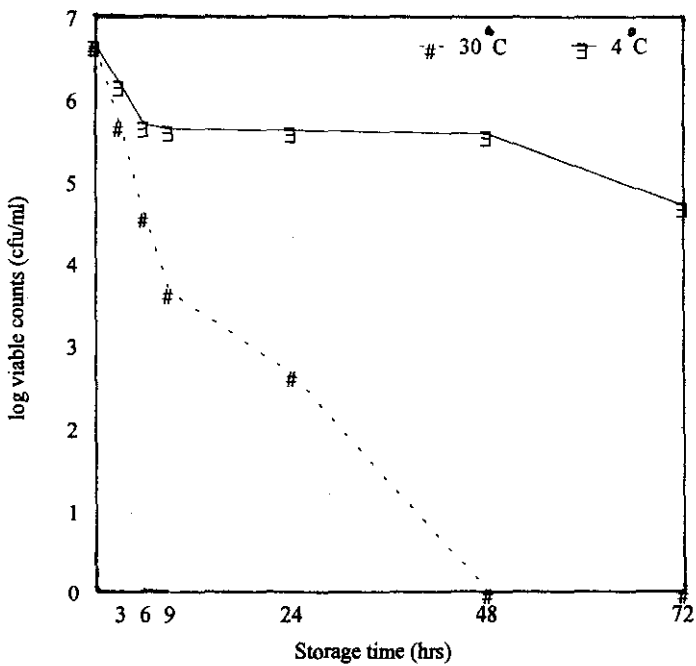


Fig 4. Growth curve of *Bacillus cereus* in soy sauce at both room and refrigeration temperatures.

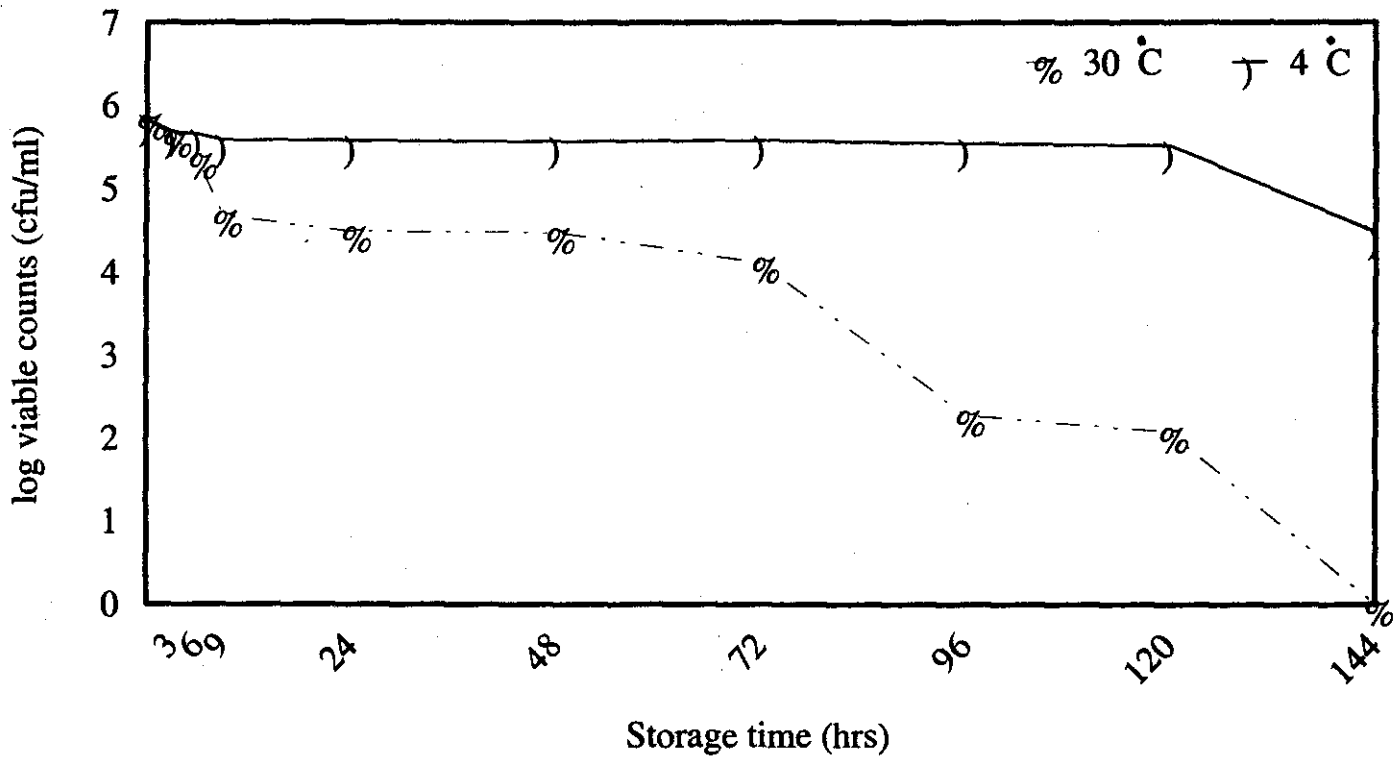


Fig 5. Growth pattern of *Salmonella typhimurium* in soy sauce at both room and refrigeration temperatures.

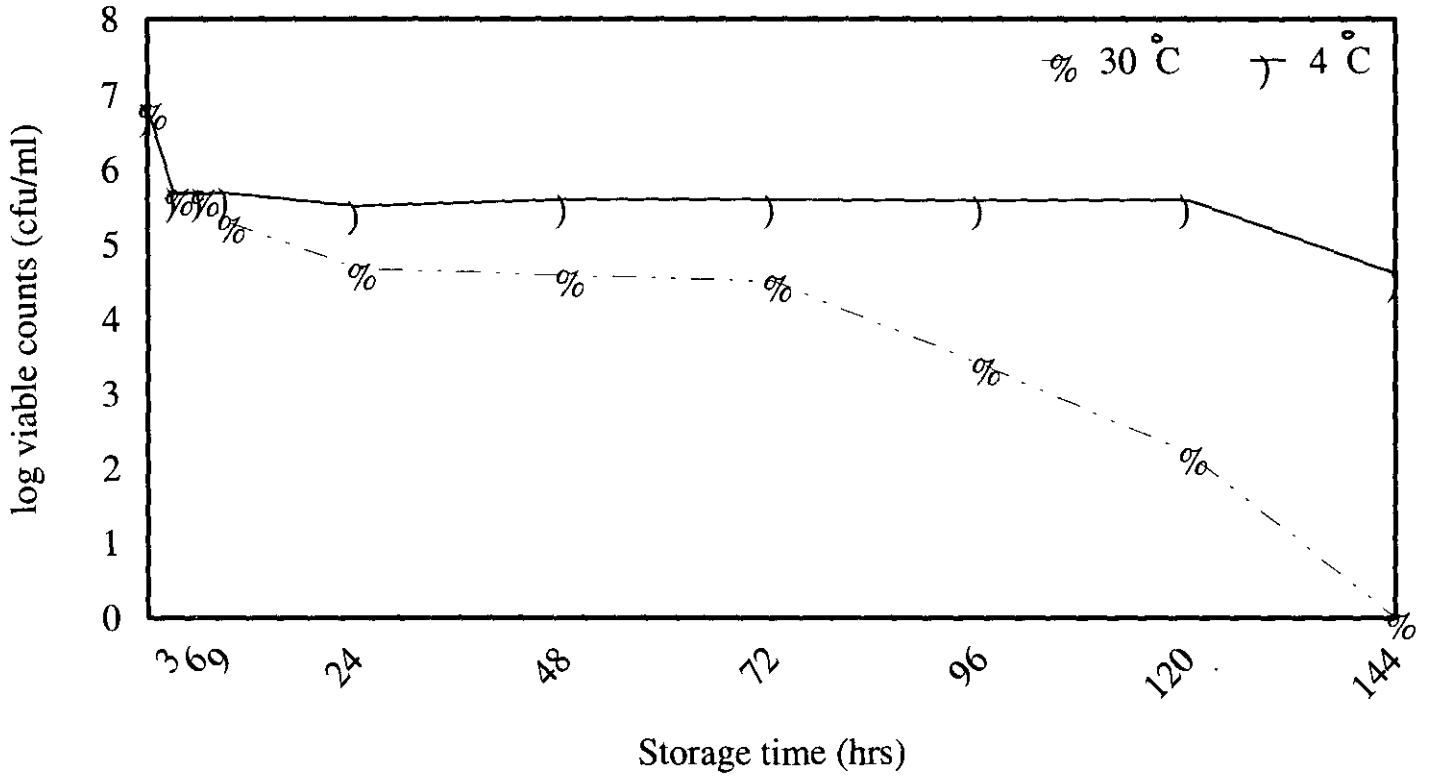


Table 1 . Bacterial inhibition zone (mm) of commercial soy sauce against some pathogens.

Bacterial strains	Inhibition zone (mm)
<i>Escherichia coli.</i>	21
<i>Staphylococcus aureus.</i>	19
<i>Bacillus cereus.</i>	14
<i>Pseudomonas aeruginosa.</i>	25
<i>Salmonella typhimurium</i>	13

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تأثير الصويا صوص المثبط للنشاط البكتيري

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تعتبر الصويا صوص من اشهر المنتجات المتخمرة والمنتشرة على مستوى العالم والتي تستخدم لإعداد بعض الوجبات مثل الشوربة و السلاطة وكذلك تستخدم لتتبيل انواع مختلفة من اللحوم (لحوم او فراخ أو سمك). تحتوى الصويا صوص على العديد من المركبات الحيوية ذات قيمة غذائية عالية ولكنها ربما تحتوى ايضا على بعض المركبات التي تحد من النشاط الميكروبي. لذا يهدف البحث لدراسة تأثير الصويا صوص المتخمرة على سلوك بعض السلالات الممرضة مثل اشريشيا كولاى و الستافيلوكوكس اورياس والسيديموناس ايروجينوزا و الباسيلس سيرس ثم السالمونيلا تيفيموريم اثناء التخزين على درجتى حرارة الغرفة (٣٠ م°) و الثلجة (٤ م°) وكانت اهم النتائج المتحصل عليها مايلي:-

- انخفاض واضح فى اعداد كلا من مثل اشريشيا كولاى و الستافيلوكوكس الي اقل من ٣٠ خلية فى الجرام خلال ٤٨ ساعة من التحضين فى الصويا صوص المتخمرة عند الحفظ فى درجة حرارة الغرفة بينما بقيت اعداد الخلايا ثابتة حتى نهاية التجربة عند الحفظ على درجة حرارة الثلجة .
- بمقارنة خلايا السيديموناس ايروجينوزا بالنوع السابق (اشريشيا كولاى و الستافيلوكوكس) فقد سجلت اعداد اقل الخلايا السابقة خلال الساعات التسع الاولى من التحضين على حرارة الغرفة ثم اختفت تماما بعد ٢٤ ساعة من التحضين بينما الحفظ على درجة حرارة الثلجة ادى الى ثبات الاعداد خلال فترة التخزين.
- اظهر ميكروبي السالمونيلا تيفيموريم و الباسيلس سيرس نتائج مشابهة فى سلوك كل منها على كل من درجتى حرارة الغرفة و الثلجة - فعلى درجة حرارة الغرفة انخفضت الاعداد ببطء الى ان اختفت تماما خلال ٦ ايام من التخزين - بينما درجة الحرارة المنخفضة تزيد من قدرة الخلايا على البقاء حية و ثابتة طوال مدة التخزين.
- مع جميع السلالات المختبرة - اظهرت الصويا صوص تأثير مضاد للبكتيريا اقوى من التحضين على درجة حرارة الغرفة مقارنة بالتحضين على درجة حرارة الثلجة
- كان التأثير المثبط للصويا صوص اقوى على ميكروب السيديموناس ايروجينوزا و اشريشيا كولاى ثم الستافيلوكوكس حيث كان قطر منطقة التثبيط ٢٥ ، ٢١ ، ثم ١٩ مم (على التوالي) - بينما تأثير المنتج على ميكروبات الباسيلس سيرس و السالمونيلا تيفيموريم كان اقلهم حيث كان قطر منطقة التثبيط ١٤ ثم ١٣ مم (على التوالي)
- اوضحت نتائج الدراسة احتواء الصويا صوص على مركبات ذات تأثير مضاد للنشاط البكتريا من المحتمل انتاجها خلال عملية التخمير لهذا المنتج او نتيجة لتركيز الملح المضاف.