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USE OF ENZYME LYSOZYME AND ANTI BACTERIAL ALLICIN FOR FOOD PRESERVATION AND THE PREVENTION OF DAMAGE

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ABSTRACT

This study aims to identify the effectiveness of enzyme lysozyme, which is extracted from the egg's albumin and the allicin antibiotic which is extracted from the garlic. It also studies their effects on some bacteria which have been obtained pure and others have been isolated in the extracts of lysozyme and allicin. Then we faced the foods which are contaminated with such species of these bacteria and their dangers on the public health. The study was conducted in two phases, the first of which included knowing the effectiveness of extracts and their effects on some sorts of bacteria which were treated by these extracts through the tests of sensitivity screening. The second phase of this study included the treatment of some types of food which are commonly circulated and speedily damaged – the treatment was with these materials to know their impact in eliminating the microbes that are found in these foods and prevent damage for a long time. We examined three types of bacteria: (*E .coli, Streptococcus* and *Salmonella*). The results of sensitivity screening, which were done in two ways: (CD-proliferation plates and confirmation way), showed the sensitivity of these microbes with rates ranging between (100%) to (33.4%) named (garlic male). While the less ones were allicin extracts that were extracted from the foreign garlic, the mixture of allicin which was extracted from the local eggs and lysozyme which was extracted from the foreign eggs with red peel. Besides, tow samples of food (milk and meat), which are commonly circulated in the governorate, were tested and treated with Allicin and Lysozyme extracts. The results showed the effectiveness of Allicin with its quality in eliminating most microbes in meat, especially *E. coli* and its effectiveness in eliminating all microbes in milk with no influence on *Salmonella*. But, lysozyme effectiveness notes varied, but it eliminated in the two samples with no influence on *E.coli*.

Key word: Allicin; food; resistant; sensitivity bacteria; garlic; egg.

INTRODUCTION

The egg's albumin contains Lysozyme, which has the ability to effect on the wall of spherical cells, which are a necessity for dying 1g. Lysozyme analyzes the walls of bacteria cells, which dye one g by intersecting the chemical bonds between N-acetylmuramic acid and N-acetyl glucose amine in the layer of Mucopeptide. Eggs are good diet, which are rich with fat, vitamins, and protein. They contain all essential amino acids. Their flavor, color, and the rates are widespread. Eggs one used in preparing several food meals In 1944, scientists could isolate the substance of (Allicin) from garlic, which is the basic material that lies behind the rare therapeutic qualities, and the smell garlic is characterized by. So garlic strengthens the body's immunity and its smell is well enough to kill the microbes, moreover, when it's small is inhaled, it mixes with blood and kills the microbes found in it. Also chewing garlic for minutes kills all mouth microbes. Garlic is herb branched with bulbs with the acrid smell of lily spicy from which the onion, leek, lily and etc. Its scientific name is *Allium allsativum* which means hot referring to sour taste (Salunkhe and Kadam, 1998). Its name in classical Arabic is foam or Thum. This name is taken from name Pharaonic (Antom). In old Arabic, its name is Shum. The ground segment of garlic consists of an onion with numerous fibrous roots and gives off the ground a number of leaves that are narrow. And stem which transcends to a height of 30 cm and ends with Bannoura tent with crimson flowers. The original place of garlic is at the Mediterranean and in neighboring countries. Garlic contains 20-27% multi-candy and 7-28 mg vitamin C, and protein 0.4% of a volatile oil consisting of poly Slvedat. It also contains Alvetosetrain.

OBJECTIVES

The objectives of the study were to be studying the inhibitory ability of lysozyme, allicin, and anti-bacterial on some microbes, especially microbe of: *Streptococcus, E. coli, Salmonella*. Using the enzyme of lysozyme and allicin antibiotic in maintaining some foods rite (milk, meat amd etc).

MATERIALS AND METHODS

Culture media: Attended all agricultural communities as instructed by the company processed and sterilized with a temperature of autoclaves at 121°C, under pressure at 15 lbs/Wang for a period of 15 minutes.

Solutions and dyes used: We brought distilled water, lysozyme with its different kinds (raw), raw allicin (local and foreign), sodium hydroxide (NaOH) and bromo cresol purple dye.

Lysozyme extraction: We chased eggs with their different kinds available in the local market in the governorate. We also took some foreign eggs with their kinds red and white (the outer peel color). Then, we took the local eggs and separated the albumin form the egg yolk. After that, we put them in

clean, dry and sterile pipes and glass containers. We covered them and transferred to the refrigerator in the laboratory unit use (Smolelis and Hartsell, 1949).

Allicin extraction: We chosed garlic with its kinds (doves) available in the local market in the province; we also took some sample of foreign garlic (the Chinese one), we took the local one with its two kinds, the first of which is known as (municipal garlic) and the second is known as (garlic male). Garlic cloves were crushed and we extracted the liquid product. The liquid was filtered by filter paper into the clean, sterile dry glass pipes and each sample was put separately to use.

Prepare (lysozyme, allicin) extracts: We prepared the allicin and lysozyme extracts and (15) extracts, 5 extracts represent the original material and the other were as a combination of these extracts.

Isolate *E. coli streptococcus* & *salmonella* bacteria: We obtained them with the help of some Sana'a hospitals like (Al-Thawra, and Seventy Hospital), and from (Benon laboratories) in Thamar.

Sensitivity screening test for *E. coli, Salmonella, & streptococcus* bacteria: It wass the way in which we used the pre-wetted filter paper in the extracts of (lysozyme & allicin). The circles were prepared which were used to develop the bacteria mentioned earlier. Also preparing the extracts of lysozyme and allicin to be put in the clean, sterillzed and day pipes. Then putting a certain amount of filter paper which was formed in the form of small disks in a diameter ranging from 0.5 cm; in each tube there were an extracts of (allicin and lysozyme). The disks we left in the tube for a period ranging between a half an hour to 2 hours to absorb a solfiaent amount of allicin and lysozyme extracts (Wilkins and Teresa, 1973; Dickert *et al.*, 1981). After isolating bacteria, we tabled the filter paper saturated with the extracts of allicin and lysozyme and placed them in different places by sterilized forceps with alcohol and flame to install on the surface by the light pressure by forceps; each one should be alone (Klančnik *et al.*, 2010). After words, incubated the grath dishes on a temperature of 37°C and for a period ranging between 24-48 hours (Coma *et al.*, 2002; Pranoto *et al.*, 2005; Kim and Yangha, 2007; Cao *et al.*, 2009; Mayachiew *et al.*, 2010).

Confirmatory tests to detect the inhibitory ability of the extracts of (allicin and lysozyme): This method was done by pouring the extracts of allicin and lysozyme on the growth after transplanting bacteria by planning to observe the inhibition. The way was the someway of (disk-proliferation), but the difference lies in that after isolation, the process of pouring a certain amount of allicin and lysozyme extracts on the growth disks and placed the dishes into the incubator to grow at a temperature of 37°C for a period and results were recorded.

Bacteriological examination of food: To conduct this process, samples of meat (minced meat), pulled 5 g of homogeneous hash in each tube. Then added 1 mL of allicin and lysozyme extracts to each tube separately. The additions were as follows: 1 mL of local allicin was added to the first tube; 1 mL of foreign allicin was added to second tube; 1 mL of local lysozyme was added to the third tube; 1 mL white foreign lysozyme was added to the fourth tube; 1 mL of red foreign lysozyme was added to 5th tube, except the 6th tube which was not treated by any extract of allicin and lysozyme extracts, however, 1 mL of distilled water was added to it. Then the samples of meat were put in the refrigerator lilt use. The samples of milk were prepared by adding 10 mL of them to each tube, total 7 tubes. Then 1 mL of allicin and lysozyme extracts was added to 5 tubes, while the milk in the 6th tube was contaminated by the fecal *E. coli* which contaminates blood. But, the 7th tube was not treated by any sample of the research samples or any bacteria. It was like (a standard sample). All samples were put in the refrigerator lilt using.

Prepare the medium (lactose broth): Lactose broth was prepared after dissolving an amount of 3.25 g in 250 mL of distilled water, adding the dye of purple bromo cresol with an amount of 0.112 g for each 250 mL of distilled water. Then lactose broth was dissolved, adding to it drops of sodium hydroxide (NaOH), in order to raise the value of pH to (7.8) on purpose to know the charge of the color in the dye as a result of low pH and at the fermentation of lactose. After that, 10 mL of lactose broth was added to all tube used. The tubes were placed, down to the sterilized lactose broth according to the instructions of the company.

Lactose fermentation test. Quantitative and presumptive test: In this part, some samples of meat were prepared and a central centrifuge period of 5 minutes for the purpose of extracting the filtrate. After sterilizing, the circle were available in the tubes, the vaccination was done by taking a point of milk and meat samples which were added to the tubes separately. After vaccinating the tubes, they were covered by a layer of sterilized was at 121°C for a period of 20 minutes. Then the cotton was put on each tube for incubating at 37°C for 24-48 hours. Directly after the incubation, the results were written down.

Confirmatory test: After noting the results of lactose fermentation and the composition of the gas, the centers were prepared. These were special for isolation of bacteria types which were expected to come out. The media used for that were as follows:

MacConcky agar, EMB, S.S. agar: After sterilization, the orcles, meida as poured on the plates and then left them to harden. During this process, the samples on which the gas was composed were prepared. Also, 1 tube was taken which represents the samples in which lactose was fermented without composing gas. Loob was taken from the tube after exposing it to the flame to sterilize and vaccinate media planning and the rest of the work was archer went likewise. After the transplant process by planning on media, the plates were put for incubating and the incubation was at different temperatures between 37- 44.5°C. The results were observed and written down.

Completed test or final test: After the growth of the colonies in the media, loob was taken to the tubes containing lactose broth and to the upside down tubes after sterilization. Incubation was

at 37°C for a period of 24-48 hours. After the incubation, the results were observed and recorded.

RESULTS AND DISCUSSION

The results in table 1, referd to the sensitivity of allicin and lysozyme extracts and their percentages. The results of sensitivity examination stated the sensitivity of these microbes for most of these extracts used in this study with rates range between (100%) for allicin extracts from garlic (garlic male), (83.4%) for lysozyme extracted from the local eggs and lysozyme extracted from the foreign eggs with white crust, the mixture of lysozyme extracted form the foreign eggs with white and red crust, the mixture of allicin extracted from the local garlic, lysozyme extracted form the foreign eggs with red peel, (66.6%) for lysozyme extracted from the foreign eggs with red peel, the mixture of allicin extracted from the local garlic, lysozyme extracted form the local eggs, the mixture of allicin extracted from the local garlic, lysozyme extracted from the foreign eggs with white crust, the mixture of lysozyme extracted from the local eggs, lysozyme extracted form the foreign eggs with white crust. (50%) for allicin extracted from the local garlic, the mixture of allicin extracted from the foreign garlic, and all kinds of lysozyme extracted. (33.4%) for allicin extracted from the foreign garlic, the tow mixture of allicin extracted from the local and foreign garlic, the tow mixture of lysozyme extracted from local and foreign eggs with red shell.

The differences can be seen through the sensitivity of allicin and lysozyme extracts. can be seen that variation in sensitivity through various forms (Fig. 1). The affected bacteria was *Streptococcus* treated by allicin extracted from the local garlic named (male garlic), but it was not affected by allicin extracted from the foreign garlic named (Chinese garlic). Regarding the effectiveness of lysozyme was high on bacteria *Streptococcus* and no the plates treated by lysozyme extracts either using lysozyme separately as mixing then together with allicin extracts. When mixing lysozyme extract extracted from the red foreign eggs with allicin extracts both foreign and local, these is growth. Regarding *E. coli* the results stated that this bacteria was affected by all extracts of allicin and lysozyme except lysozyme local extract when mixed with the extracts of allicin both foreign and local. But, mixing Lysozyme extract with allicin foreign extract states the former results (Disk diffusion way) (Rees *et al.*, 1993).

For *Salmonella* bacteria, the result stated the effectiveness of lysozyme extract on *Salmonella*, however, there was no growth for this bacteria when using the two extracts of lysozyme the foreign extract both red and white. But mixing lysozyme extracts together and with allicin extracts stated different result; there was no growth when mixing lysozymen foreign extract (the white one) with allicin foreign extract. The sample in which allicin extracts with their types both local and foreign with lysozyme. This mean that the mixture of allicin extracts and lysozyme's did not gave effectiveness. The rate of the *Salmonella*, the results showed the effectiveness lysozyme extracted from the egg domestic as well lysozyme extracted form the foreign eggs quality red and white and these results match the results obtained from Facon and Brent (1996) in a previous study has the effect lysozyme and substance EDTA factors discouraging *Salmonella* bacteria and the effectiveness of the enzyme made clear lysozyme *Salmonella*. Some results did not state the effectiveness of allicin extracts by using them separately or mixing them together on *Salmonella*. Through the results, it was stated that there was on effect of allicin extracts on bacteria *E. coli*. These results correspond with Rees *et al.*, (1993); Breithaupt-Grögler *et al.* (1997) which stated that was an effect on bacteria *E. coli*. Lysozyme effect on bacteria *Salmonella* was similar to Facon and Brent (1996) study which stated that there was effectiveness on *Salmonella.*

Through the experiments conducted on allicin and lysozyme with their phases (first and second), the experiment stated that allicin has an effect on *E.coli* and limited on *streptococcus*. On the other hand, it was not rated that it has an effect on *Salmonella*, although, it has strong effectiveness in eliminating most microbes found in most. Moreover, it has strong effectiveness in eliminating all microbes which were found in milk when treated with these extracts (local and foreign). Results have shown a sample of milk, which was known to milk from the appropriate sources for the growth of various microorganisms and which ones causef damaged.

The results that extract of sllicin is highly effective did not prove ferment lactose or gas in the pipeline that treated milk which extract allicin remained colored as color pigment blue dark, and was also confirmation of the results through the implant on different media which did not show any colonies in central EMB , MacConcky Agar and S.S. Agar.

The extract of lysozyme has demonstrated the effectiveness of the approach of allicin and extracted it of lysozyme extracted derived from local eggs and egg Lysozyme extracted from the outside (white) has effective in the inhibition and did not show any planting colonies when the central S.S. Agar. The lysozyme extracted from white outer (red) did not have an impact notes ferment lactose with gas formation and colonies appeared in various media used.

Through studies conducted on extracts lysozyme and allicin stages I and II experience has shown the influence of these substances uneven and that was allicin has an effect on *E.coli* effectively and limited *Streptococcus* was observed effect on *Salmonella*, and effectiveness of high eliminate most microbes found in meat. This corresponds to what was stated in and its effectiveness concluded in eliminating all of the microbes in the milk counter when treated in this local and external quality, compared with the standard sample (Table 2) and effectiveness in eliminating bacteria *Salmonella* were found in milk and this corresponds to what Lord's (Breithaupt-Grögler *et al.*, 1997). It was identical to a previous study conducted for (Rees *et al.*, 1993), which explained all to the effectiveness of Allicin *Salmonella*.

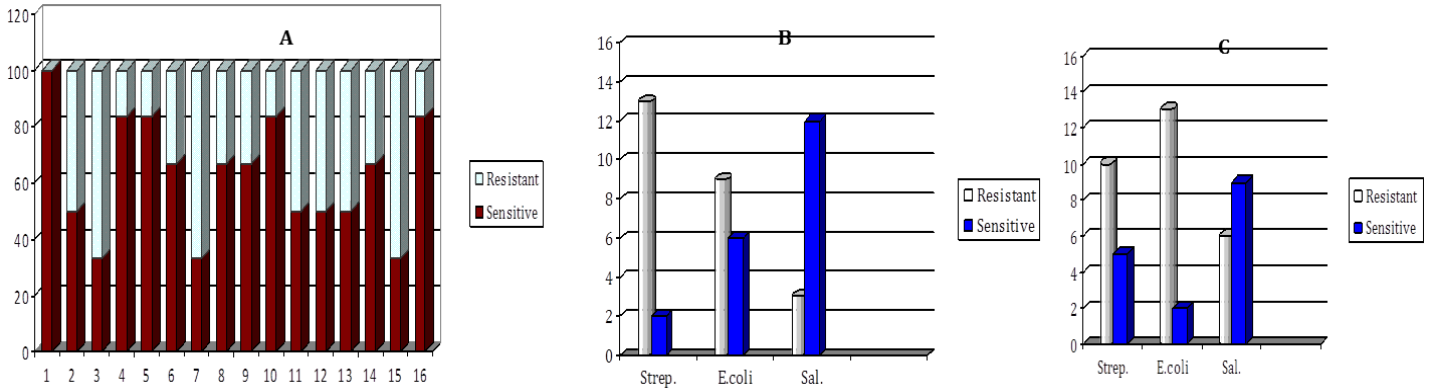

Figure 1: sensitive bacteria extracts (lysozyme and allicin method of disks spread) sensitive bacteria extract (A), the sensitivity of bacteria to extract (Allicin, Lysozyme) (B) and sensitive bacteria extracts (lysozyme and allicin; confirmation method).

Table 1: The percentage of sensitivity and resistance of bacteria isolated Extract (allicin and lysozyme):

Extracted	No.	The test No of times	Sensitivity	%	The No of resistance	%
Local allicin (male garlic)	1	3	3	100	-	-
Local allicin	2	3	3	50	3	50
Foreign allicin	3	6	2	33.4	4	66.6
Local lysozyme	4	6	5	83.4	1	16.6
Foreign lysozyme (white)	5	6	5	83.4	1	16.6
Foreign Lysozyme (red)	6	6	4	66.6	2	33.4
Allicin foreign and local	7	6	2	33.4	4	66.6
The local allicin and the local lysozyme	8	6	4	66.6	2	33.4
The local allicin and the foreign Lysozyme (white)	9	6	4	66.6	2	33.4
The local allicin and the foreign lysozyme (red)	10	6	5	83.4	1	16.6
The external allicin and the local lysozyme	11	6	3	50	3	50
The external allicin and the external Lysozyme (white)	12	6	3	50	3	50
The external allicin and the external Lysozyme (Red)	13	6	3	50	3	50
Lysozyme local and external (white)	14	6	4	66.6	2	33.4
Lysozyme local and external (red)	15	6	2	33.4	4	66.6
The external Lysozyme (white and red)	16	6	5	83.4	1	16.6

Table 2: The fermentation of lactose, and the product gas in a sample of meat and milk

Tube No	Meat-Treatment Sample	Fermentation of lactose	Begas	Fermentation of lactose	Begas
		Meat		Milk	
1	Local allicin	+	–	–	–
2	Foreign allicin	+	–	–	–
3	Local lysozyme	+	+	–	–
4	Foreign Lysozyme (white)	+	+	+	+
5	Foreign Lysozyme (Red)	+	+	+	+
6	Distilled water	+	+	+	+

CONCLUSION

1. High efficiency of the Lysozyme enzyme on *Streptococcus* and *Salmonella*.
2. The effectiveness of some food; the local one was more effective among them.
3. The effectiveness of allicin in eradicating *E.coli* in all transactions.
4. The ineffectiveness of allicin on bacteria *Salmonella* isolated from chicken livers wer found in meat and its effectiveness on the same bacteria wer found in milk
5. The effectiveness of allicin extracted from the local garlic named (male garlic) and its ineffectiveness in the other treatments with its local and foreign types on bacteria *Streptococcus*.
6. The possibility of using allicin and lysozyme extracts in some foods, quickly damaged because of their impact on many microbe existing in foods causing damage, as well as food poisoning.
7. The effectiveness of Lysozyme foreign extract (the red one) on microbes when treating foods whith it.

RECOMMENDATIONS

1. Studying the possibility of using lysozyme enzyme in the filed, of food processing such as improving conservation
2. meat with its kinds.
3. Extraction and purifying the components of both lysozyme and allicin and studying their effect on microbes which cause food damage and diseases widely.
4. Using allicin pure in treating meat, milk and other foods, especially those which coliform increases and those that are kept for a long time because allicin is effective against microbes. It is of high therapeutic and healthy, importance with using lysozyme with foods in which largely *Salmonella* is found especially meat and us derivatives for lysozyme enzyme has strong effectiveness on *Salmonella*.
5. Doing a similar future study all around the republic to study the effectiveness of allicin *on Salmonella* isolate separately to try using allicin pure to see its impact on these isolates then specifying the sensitive ones to the counter and the resistance. This study is way important because *Salmonella* is a microbe among microbes which are the most commonly spread in the Republic of Yemen.

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