Study of The Inhibitory Activity of The Alcoholic Extract of Licorice Plant Glycyrrhiza Glabra and Its Effect on Prolonging The Storage Period of Manufactured Biscuit

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Abstract

The study was conducted to obtain the alcoholic extract of the licorice roots plant and to test its effectiveness against diagnosed bacterial isolates. This research was conducted at the Food Contamination Research Center / Department of Environment and Water / Ministry of Science and Technology. The results showed the following: 1-80% ethyl alcohol was used to obtain the alcoholic extract using a saxolite device, and the activity of the extract was tested against four bacterial isolates diagnosed in the Food Contamination Research Center, which included two gram-negative isolates of Escherichia Coli and Pseudomonas aeruginosa, and two gram-positive isolates of Bacillus Cereus and Staphylococcus aequastreus by a method, in addition to yeast by Diffusion method. The study included two treatments of biscuits with alcoholic extract (A) at a concentration of 10% and compared to the control treatment (B) without any adding and for preservation periods (1, 4, 8, 15, 22) days, and microbial tests were conducted for the biscuits. The alcoholic extract showed a lethal activity to microorganisms and no bacterial cell appeared in the biscuit samples except for the incubation period (22) days for storing biscuits, which recorded the presence of fungi (2) cells/ml. Conducting sensory evaluation of the treatments (A, B) for biscuits where there were no significant differences (P<0.05) for sensory attributes except for flavor and flakes of biscuits.

Keywords: Glycyrrhiza, Manufactured, Bacterial, food.

1. Introduction

The need has grown in the past few years to use natural food additives that act as antimicrobials to meet the consumer's desire and meet the increasing nutritional requirements of humans and to stay away from the use of what is industrial for food ingredients, as well as to secure the health and safety of humans. There are plants in nature that contain biologically active chemical compounds that can be used in the field of food preservation technology, as they are used in food manufacturing in order to extend their shelf life [1]. Since the basic measure in evaluating the suitability of baked products is their freedom from microbial contamination, where the appearance of bacteria (Escherichia coli, staphylococcus aureus) is an important criterion for contamination and assessment of validity for human consumption or not. Fungi have an important role in contaminating food, including baked goods, especially drought-tolerant fungi (Aspergillus fungus), which causes some of its types of dangerous effects on human health. The presence of these types of bacteria and fungi above the permissible limit is an indication that food is not acceptable [2]. Licorice contains many effective chemical compounds (phytochemicals) and it is one of the important plants on the medical, nutritional, and economic level [3]. It contains glycyrrhizin, which is the main compound found in the roots. Many researches have been conducted to benefit from it at the nutritional level, it characterized by its sweetness. The sweet taste is the most acceptable by many age groups [4]. Also, glycyrrhizin has high thermal stability, which makes it suitable for manufacturing heat-treated foods and it possesses compounds that have antimicrobial activity [5]. The plant-derived coumarin is effective against fungi, which increases the importance of using the licorice plant in food preservation [6]. Therefore, the current study aimed to:

1. Knowing the anti-alcoholic effect of licorice extract on microorganisms (available isolates of bacteria and fungi) in prolonging the shelf life of biscuits.
2. Comparing the sensory traits of laboratory-made biscuits with the control sample through sensory evaluation.
2. Materials and Methods

2.1 Sample collection

The licorice plant was obtained from the local markets in the city of Baghdad. Licorice roots are cut and milled with an electric Hermle grinder to obtain a homogeneous powder. Store in clean glass containers until use.

2.2 Diagnosis of the licorice plant

The plant was diagnosed by the Herbarium of the College of Science - University of Baghdad, and the sample was classified by the type Gaycyrrhiza glabral and the family Fabaceae.

2.3 Preparation of the alcoholic extract of the licorice plant

To prepare the alcoholic extract of licorice using the method [7] with some modifications as follows: Extraction was conducted by placing 50 g of licorice powder in a thimble, which was placed in a Soxhlet continuous extractor. 80% ethyl alcohol was used with an amount of 250 ml alcohol and at a temperature of 80 °C, and then the extract was taken and concentrated using a rotary evaporator at a temperature of 45 °C, and the model was placed in the oven at a temperature of 37 °C for 10 hours to obtain a dry powder, and it was kept in the refrigerator until use.

2.4 Efficacy of licorice extracts on experimental bacteria and yeasts

The Well diffusion method was used to study the effect of the alcoholic extract of licorice plant on bacteria and yeasts [8]. Where the agar medium feeding the test bacteria was inoculated by diffusion of 0.1 of the bacterial suspension containing 810 x 1.5 cells/ml in comparison with McFarland solution and 5 mm diameter holes were made on the surface of the culture medium by a cork drill. Test yeast colonies were inoculated with Sabaroid dextrose Agar medium with a sterile swab, of a yeast suspension containing 810 x 4 cells/ml. The prepared concentrations of the alcoholic extract of licorice powder with concentrations (1,2,5,10,20,30)% of the alcoholic extract, respectively, were placed at 0.1 ml in the hole, with a control hole containing diluted ethyl alcohol at a concentration of 85% sterile as a control for the alcoholic extract. The dishes were left to harden [9]. Then the dishes were incubated for (2±24) hours in the incubator at a temperature of 37°C, and the diameter of the inhibition zone around each hole was measured.

2.5 Manufacturing of laboratory biscuits (the basic recipe)

Materials:
White flour 100 g
Solid fat 22.7 g
salt 2.7 g
Baking powder 4.9 g
Milk 73.6 g

2.6 The work method

The biscuits were prepared in the laboratory (with some modifications in the weights of the materials used) according to the following steps:

1. Sift the flour, salt, and baking powder together into a mixing bowl. The oven temperature is regulated at 218°C.
2. add the fat to the dry ingredients.
3. Then the liquid milk was added to the dry ingredients, then the ingredients were mixed well with the fork and several times (about 30 times) until the dough became homogeneous.
4. Spraying the wooden board with flour and spread the dough with a thickness of 0.5 pieces in a circular biscuit mold with a diameter of 5 cm.
5. Put the biscuits in the not greased mold using a special spatula knife and leave a distance of 1-1.5 cm between the biscuit pieces until the color becomes golden [10].
Table 1. Coding of treatments and concentrations of Additives materials.

| Baked product | Treatments | Additives materials       |
|---------------|------------|---------------------------|
| Biscuit       | A          | 10% alcoholic extract     |
|               | B          | Control treatment without any addition |

The biscuits were made and treated with alcoholic extract, and the following steps were taken:
1. Conducting microbial and sensory tests on the day (0).
2. Keep the biscuits in the refrigerator at a temperature of (4) °C.
3. Microbial tests were repeated on the day (4), (8), (15), and (20).

2.7 Microbiological tests

Microbiological tests were conducted for the biscuit samples, which included a total count of aerobic bacteria, coliform bacteria, salmonella, and yeasts. According to the standard specification issued by the Iraqi Central Organization for Standardization and Quality Control No. 2270/10 for the year (2006) [11], which indicates that the number of cells of *Staphylococcus aureus* and *E. coli* bacteria when $1 \times 10^2$, cell to be a good product for human consumption, and when $1 \times 10^{10}$. A cell for the product to be acceptable. While the number of salmonella bacteria cells is required to be zero for the product to be suitable for human consumption but requires the preparation of molds and yeast cells when $1 \times 10^{-2} - 1 \times 10^{-4}$ cell for the product to be acceptable.

2.7.1 Calculation of the total number of aerobic bacteria

The 1mL of the dilution was transferred to a Petri dish, and 20 mL of sterile SPC agar medium (SPC) cooled to (45-48) °C was poured into sterile dishes. The dishes were quietly rotated, left until the medium solidified, and then incubated for (24 ± 2) hours at a temperature of 37 °C.

2.7.2 Detection of salmonella bacteria

Transfers 0.1 ml of the mixture to 10 mL of liquid tetraphionate medium in a test tube, incubate for (24 ± 2) hours, then spread 0.1 mL of it onto xylose-lysine-deoxycholate (XLD) agar media using a sterile glass diffuse and incubate the dishes for (24 ± 1) 2) an hour at a temperature of 37°C.

2.7.3 Detection of coliform bacteria

MacConkey Broth (MPN) liquid medium, containing an inverted Durham tube, was used to inoculate the broth in the amount of (1.5, 0.5 ml) of the sample to be examined and incubated for (18-24) hours at a temperature of 37°C.

2.7.4 Detection of yeasts

Sabouraud dextrose agar (SDA) medium was used for the detection of yeasts. The medium was prepared according to the manufacturer's instructions, and the medium was added into sterile Petri dishes. It was left until the medium solidified, then the media was inoculated with the mixture and incubated, at a temperature of 25-28 °C for (3-5) days, and the developing colonies were counted.

2.7.5 Sensory evaluation

Sensory evaluation was conducted at the Ministry of Science and Technology - Department of Environment and Water - Food Pollution Research Center. Samples of laboratory-made biscuits were Sensory evaluated by 30 specialized assessors according to the sensory evaluation form approved by Food and Nutrition of Kansas State University (1975) and according to The sensory assessment score of 7 according to the Basket Sensory Assessment Form [12].

2.8 Statistical analysis

The statistical program [13] was used to analyze the statistical data to study the effect of different treatments on the studied traits according to a completely randomized design (CRD) and the significant differences between the means were compared with the least significant difference test (LSD).
3. Results and Discussion

3.1 Effectiveness Test of Licorice Extract

Four types of bacteria were used in this study: Bacillus cereus and Staphylococcus aureus, which are gram-positive, while Escherichia coli and Pseudomonas aeruginosa are gram-negative. Note that the total bacterial number used in the experiment comparing the inhibitory activity of licorice plant extracts was 1.5410/ml with 2ml xl. McFarland solution and after serial dilution to obtain turkey, these microorganisms are among the contaminants that cause food spoilage and are considered a common cause of diseases affecting humans and animals [14].

3.1.1 Inhibitory activity of the alcoholic extract of licorice plant in a number of bacterial isolates and yeasts

The inhibitory activity of the alcoholic extract of licorice plant at concentrations (1, 2, 5, 10, 20, 30) was tested to know the effect of the extract on bacterial isolates and yeasts diagnosed as in Table (2). The alcoholic extract with a concentration of 1% had the lowest inhibitory activity, and the diameter of inhibition was 8 mm against Bacillus cereus. While the highest inhibition diameter was 18 mm at a concentration of 30%. The alcoholic extract showed an effect against Escherichia coli bacteria at a concentration of 10%, and the diameter of inhibition was 7 mm. The highest diameter of inhibition was 10 mm at a concentration of 30%. The alcoholic extract of all concentrations did not show any inhibitory effect against Staphylococcus aureus Pseudomonas aeruginosa bacteria. As for its effect on yeasts, it possesses an inhibitory ability of 10 and 20% diameter against yeast 6 mm, while the inhibitory activity of alcoholic extract at a concentration of 30% against yeast reached an inhibitory diameter of 8 mm. 10% has a lower inhibitory effect on yeast than on bacteria.

Table 2. Inhibitory activity of alcoholic extract of licorice plant in a number of bacterial isolates and yeasts.

| Alcoholic extract% | Yeast | Pseudomonas aeruginosa | Escherichia coli | Staphylococcus aureus | Bacillus cereus |
|--------------------|-------|------------------------|------------------|----------------------|---------------|
|                    |       |                        |                  |                      |               |
| 1                  | 0±0.00| 0±0.00                 | 0±0.00 b         | 0±0.00               | 8 ±0.06 c     |
| 2                  | 0±0.00| 0±0.00                 | 0±0.00 b         | 0±0.00               | 9 ±0.04 c     |
| 5                  | 0±0.00| 0±0.00                 | 0±0.00 b         | 0±0.00               | 10 ±0.08 bc   |
| 10                 | 6 ±0.05| 0±0.00                 | 7 ±0.06 a        | 0±0.00               | 13±0.08 abc   |
| 20                 | 6 ±0.05| 0±0.00                 | 9 ±0.08 a        | 0±0.00               | 15 ±0.11 a    |
| 30                 | 8 ±0.09| 0±0.00                 | 10 ±0.08 a       | 0±0.00               | 18 ±0.09 a    |
| values LSD         | 2.770 | NS                     | 3.76 *           | NS                   | 5.21 *        |

The averages with different letters within the same column differ significantly between them. (P<0.05*).

Table (2) showed the effect of the licorice plant on bacteria and yeasts. The reason can be due to the alcoholic extract containing many effective groups in a plant due to the presence of glycosides, resins, saponins, flavonoids, and terpenes, which is due to the inhibitory activity of the different isolates, and that the alcoholic extract works against bacteria and this Corresponds to the study [15] who stated that this activity is due to the presence of saponins, alkaloids, and flavonoids, and this is also consistent with the study [16]. The plant contains flavonoids, which have antimicrobial activity and the highest inhibitory effect against gram-positive bacteria Staphylococcus aureus, Bacillus cereus sp. In addition to the study of [17] confirms that the substance G. glabra present in the licorice plant has activity against Staphylococcus aureus. Also, the alcoholic extract showed strong activity against microbes except for Pseudomonas aeruginosa bacteria, and this result was consistent with the study [18], and it differs from the study of [19].

3.2 Inhibitory activity of the alcoholic extract against bacteria of the licorice plant to increase the shelf life of biscuits

Table (3) shows the inhibitory activity for the growth of bacterial isolates in the biscuit model treated with alcoholic extract, where the alcoholic extract showed excellent and was gained against the growth of bacterial cells. .4,1) days in a row at a temperature of (4)°C. While the biscuit samples that were not treated with licorice plant extracts, which is the control treatment, it was noted that the numbers of cells formed for bacterial colonies contaminated with biscuits appeared, which amounted to (510X125 cells/mm) during the preservation period (22) days, while there was no growth of colonies of bacterial cells during the preservation period (1,4,8,15) days at a temperature of (4)°C. Thus, and through Table (3), the storage results of the samples showed that there were no significant differences at the level of probability (P<0.05) for all treatments during storage periods (22,15,8,4,1) days. Bacterial infection when treated with alcoholic extract during 22 days of preservation, the significant value reached (22.87) for the preservation period of 22 days. The results did not show a significant difference
between treatments with alcoholic extract compared with the control model for a preservation period of (1, 4, 8, 15, 22) days and it amounted to (0), for the testing for salmonella and coliform bacteria.

Table 3. Inhibitory activity of the alcoholic extract against bacteria of licorice plant to increase the shelf life of biscuits.

| Material     | First day | The fourth day | Eight day | Fifteenth day | Twenty-second day |
|--------------|-----------|----------------|-----------|---------------|-------------------|
|              | SPC 10    | salmonella    | Coli      | salmonella    | Coli              |
| Alcoholic    | 0 ±0.00   | 0 ±0.00       | 0 ±0.00   | 0 ±0.00       | 0 ±0.00           |
| control      | 0 ±0.00   | 0 ±0.00       | 0 ±0.00   | 0 ±0.00       | 0 ±0.00           |
| LSD value    | NS        | NS             | NS        | NS             | 22.87 *           |

The averages with different letters within the same column differ significantly between them. (P<0.05*).

Through Table (3), the results showed that the estimation of the bacterial content in the biscuit samples and there were differences in the number of bacteria between the different types of treatments. on all microorganisms [20] It was mentioned [21] that the total number of Escherichia coli bacteria in the final product after baking is 0.0*310 cells/gm. This is consistent with the study [22]. The presence of Escherichia coli in the final product is a source of health concern, as well as indicates deficiencies in terms of food hygiene control. The total number of enteric bacteria allowed to be present in bread and pastries ranges between 10-100 cells / g [23] As for the number of colonies present in treatments for baking and storage of biscuits and all types of microorganisms, it can be mainly due to pollution resulting from the air or some of the tools used in manufacturing and preparation in addition to surfaces during cooling [24]. Including the dough [25]. The addition of licorice plant extracts has shown effectiveness against microorganisms due to the licorice’s antimicrobial activity [26]. The licorice extract showed inhibitory activity against gram-negative and gram-positive bacteria. The distinctive activity is due to the presence of the active compounds present in the plant extract where it has antimicrobial activity [5]. In addition to the studies that proved that moisture affects baked foods, they indicated that a higher level of moisture content indicates a short shelf life because it encourages the growth of microbes, which leads to spoilage. This is consistent with the study of [27] in addition to the study of [28]. The results of the studies also demonstrated that the alcoholic extract has an effect against bacteria and this is consistent with the study [29]. And the study showed that biscuits have a better storage capacity, and this is consistent with the study [12].

3.3 Inhibitory activity of alcoholic extract against yeasts of licorice plant to increase the shelf life of biscuits

The results in Table (4) show that there was no growth in the numbers of yeasts for the biscuit product treated with alcoholic extract at 10% concentration during the different storage periods represented in 22 days of preservation. As for the control treatment, there was no growth in the number of yeasts during the preservation period (4.1) days, while the preservation periods were (22,15,8) days, respectively. It showed a number of yeasts estimated at (24,154,306) cells/mm, respectively, and thus the number of yeasts increased during the increase in the storage period, and it became clear that there was a significant difference at the level of significance (P<0.05). 8 to day 22 of storage, as the significant values of the biscuit product, were (72.08, 61.37, 23.81), respectively.

Table 4. Inhibitory activity of alcoholic extract against yeasts of licorice plant to increase the shelf life of biscuits.

| Material     | First day | Fourth day | Eight day | Fifteenth day | Twenty-second day |
|--------------|-----------|------------|-----------|---------------|-------------------|
|              | SPC 10    | Salmonella | Coli      | Salmonella    | Coli              |
| Alcoholic    | 0 ±0.00   | 0 ±0.00    | 0 ±0.00   | 0 ±0.00       | 0 ±0.00           |
| Control      | 0 ±0.00   | 0 ±0.00    | 0 ±0.00   | 24 ±0.63      | 154 ±8.02 a       |
| LSD value    | NS        | NS         | NS        | 51.75 *       | 82.26 8           |

The averages with different letters within the same column differ significantly between them. (P<0.05*).

It is clear from tables (4) that it agrees with the study of [30] that fresh bread and pastries do not contain any yeast growth and that contamination of bread and pastries with them occurs after a short period of production operations, especially pollution that occurs due to machinery, equipment, and improper handling. The number of molds allowed to be present in bread and pastries ranges between 10-310 cells/g [31]. This is consistent with the results of the study referred to by [32], where they found that the large percentage of pastry contamination in India is due to molds. In addition to that, the thin-slice pastries with
water activity 0.71 - 0.89 and pH (4.62-8.82) are exposed to pollution from before molds and drought-tolerant yeasts, due to the appropriate pH and temperature.

3.4 Sensory evaluation of laboratory bast

Table (5) shows the sensory evaluation of the biscuit samples that were treated with alcoholic licorice extract compared to the control treatment without any addition. The treatments treated with licorice extracts got the value (5.8), while the control treatment got the value of the appearance trait at (6.0). With regard to the tissue trait, there are no significant differences at the level of significance (P<0.05) for both treatment and control groups (5.4), (5.5), respectively. As for the traits of softness, the table indicates that there are no significant differences at the level of significance (P<0.05) between the different treatments (5.5, 5.3). With regard to the flavor traits, the control treatment obtained the highest value (5.5), while for the alcoholic extract was (5.0). As for the lamellar trait, the control treatment reached the highest value (5.3), while the alcoholic extract treatment reached (4.9). With regard to the color trait, there are no significant differences (P<0.05) between treatments and the control group (6.2,6.0), respectively. It is clear from Table (5) that there were no significant differences (P<0.05) between the treatments and the control group for the sensory traits of appearance, texture, softness, and color. However, there was a slight change in the three treatments about flavor and flakiness traits.

Table 5. The effect of different treatments on the results of the sensory evaluation of the laboratory biscuits.

| Treatments   | Appearance | Color   | Flakiness | Flavor  | Softness | Texture | Total       |
|--------------|------------|---------|-----------|---------|----------|---------|-------------|
| Alcoholic    | 5.8 ±0.15  | 6.0 ±0.17| 4.9 ±0.02 | 5.0 ±0.04| 5.3 ±0.11| 5.4 ±0.12| 32.4 ±0.59  |
| Control      | 6.0 ±0.22  | 6.2 ±0.23| 5.3 ±0.04 | 5.5 ±0.11| 5.5 ±0.08| 5.5 ±0.12| 34.0 ±0.74  |
| LSD value    | 0.407 NS   | 0.379 NS | 0.382 *   | 0.495 *  | 0.488 NS | 0.369 NS | 1.993 NS    |

The averages with different letters within the same column differ significantly between them. (P<0.05*).

The highest score awarded for each characteristic is 7, the lowest score awarded for each characteristic is 1, where 7 = excellent, 6 = very good, 5 = good, 4 = average high, 3 = average, 2 = approval, 1 = very poor.

Conclusions

1. The possibility of using licorice root extracts to discourage microorganisms contaminating baked goods.
2. The possibility of using the alcoholic extract at a concentration of 10% to increase the shelf life of baked products.
3. The possibility of producing baked goods with licorice extract added without affecting the taste and softness.

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