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Research Article

Study Effect of Peppermint Extract on Microorganisms Isolation and Identification from Pizza

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Abstract:

This inquiry is centred on two pizza-related products. Frozen pizza was more popular in this culture than fresh pizza. Of the total colonies, 43 (or 86% of the total) were β-hemolytic, while 16 (or 32% of the total) were γ-hemolytic, according to the data. In this particular experiment, the G-ve and G+ve bacteria were dyed in distinct ways. When cultivated on blood agar, the selected bacteria for this investigation exhibited β-hemolytic activity. Nineteen percent were G+ve and thirty-four percent were G-ve, as shown in Figure (3-2). Bacteria in this experiment were shown to be sensitive to a peppermint extract diluted 10-2. The experiment was conducted using fresh peppermint. Following the addition of the extract to the wells, the inhibitor zone diameter was evaluated using the well diffusion method. When compared to the aqueous extract (resuspension), the methanolic extract had less antimicrobial action. One possible explanation is that the extract doesn't have good diffusion properties in the agar. Another possibility is that the solvent alters the properties of the active chemicals present in fresh plants.

Keywords: Peppermint, Isolation, Microorganisms, Pizza

Introduction:

The classic pizza recipe calls for yeasted flatbread, tomato sauce, and cheese baked to perfection in an oven. A variety of meats, veggies, and condiments are typically placed on top. A Latin book from Gaeta, Central Italy, dates back to the 10th century, when the term was first recorded (Maiden, 2003). Pizza as we know it today originated in Naples, Italy, but its variations have since spread over the globe (Miller, 2012) At Italy's request, the European Union designated Neapolitan pizza as a Traditional Speciality Guaranteed dish in 2009. (OJEU, 2010; ITA, 2010) The True Neapolitan Pizza Association, or Associazione Verace Pizza Napoletana, is a Naples-based non-profit that has been around since 1984. The "genuine Neapolitan pizza" is promoted and protected by it (AVPN, 2015). Pizza is a popular fast food item in North America and Europe, and it can be bought either whole or in portions, and it can be frozen. To cook them, you can use one of several different kinds of ovens. Sanders and Sanders (1992) noted that pizza ingredients are also used to make stromboli and calzone, two comparable cuisines. Those working in infection control around the world are understandably worried about the ever-increasing prevalence of bacteria and viruses that are resistant to several drugs. This situation has led to the resurgence of diseases that were previously under control and has significantly increased the

frequency of opportunistic and chronic infection cases in developing nations, as well as driving up the expense of medication regimens (Sanders and Sanders, 1992). (Dhwani 2014; Fernandez 2003)

2.1.Materials

2.2.1. prodect collection:-

One home pizza and one market pizza were retrieved from the Halla company, and they were kept for a period of four months, from October 10, 2016, to January 16, 2017. Data regarding their (date, composition, and carlic use). The pizza and pie farce. Gralic was not included in farcing.

2.2.2. Preparation of culture media:

The following general culture media was prepared using the normal methods: nutrient agar medium, nutritional broth medium, and Mueller-Hinton agar medium. The ready-made culture media was prepared according to the manufacturer's instructions. All of them were utilised in the right tests.

2.2.3. Brain heart infusion-glycerol broth medium:-

To make this medium, 95 millilitres of BHI broth was mixed with 5 millilitres of glycerol and then autoclaved to kill any bacteria. Its application was in a test to identify the formation of bacteriocin (Forbes et al., 2007).

2.2.4 Stain:

Following the procedures outlined by Benson (2001), Gramme stain was used to distinguish between Gram-negative and Gram-positive bacteria, as well as to determine their morphology and arrangement.

2.2.5 Peppermint and aqueous extract preparation

We bought peppermint bulbs fresh from the AL-Hilla market. The edible part of the cloves was extracted by separating and peeling them. Using a Waring blender, 50 grammes of the edible portion were diced and mixed with 100 mL of autoclaved water. A crude aqueous extract containing 500 mg of peppermint/mL was obtained by passing the homogenate through a 25-mm pore-size filter (Millipore, St. Quentin, France). The specimen was preserved in a sterile vial and kept at 4¡C until it was needed.

2.2.6 Peppermint and Alcoholic extract preparation

We bought peppermint bulbs fresh from the AL-Hilla market. The edible part of the cloves was extracted by separating and peeling them. Using a Waring blender, 50 grammes of the edible portion were diced and mixed with 100 mL of alcohol. A crude aqueous extract containing 500 mg of peppermint/mL was obtained by passing the homogenate through a 25-mm pore-size filter (Millipore, St. Quentin, France). The specimen was preserved in a sterile vial and kept at 4¡C until it was needed.

2.2.7.Well diffusion method.

To follow this procedure, we made sure to cut evenly spaced wells (6mm) in the Muller-Hinton agar plates. Then, we infected the plates by dipping cotton swabs into a scrow tube that contained a bacterial suspension and streaking them over the plates. Afterwards, 0.1 ml of each antibiotic's produced concentration was added to Muller-Hinton agar wells, and the plates were then incubated at 37oC for 24 hours. By gauging the inhibitory zone surrounding the wells for each concentration, the antibiotics' susceptibility was ascertained (Norrel and Messely, 1997; Hangnga et al., 2002).

3.1 Results:

3.1.1. Isolation and Identification of bacteria:

Two pizza-related goods are the focus of this investigation. The culture was favourable towards frozen pizza, but unfavourable towards fresh pizza .

The accompanying table (3-1) shows the findings of the positive culture (frozen pizza) that appeared after taking 1 gramme of pie and farcing from each sample and culturing them on various medium.

Table (3-1) CFU/gm of microorganisms

TYPE PIZZA	CFU/GM OF MICROORGANISMS				
	MacConky agar	Blood agar	Potata dextrose agar	Malt agar	Nutrient agar
farcing	$6*10^2$	$26*10^2$	$26*10^2$	$17*10^2$	$16*10^2$
pie	$2*10^2$	$33*10^2$	$1*10^2$	$9*10^2$	$53*10^2$

3.1.2 Blood hemolysis:

The results showed that out of the total number of colonies, 43 (86%) were β-hemolytic, whereas 16 (32%) were γ-hemolytic, as shown in figure (3-1).

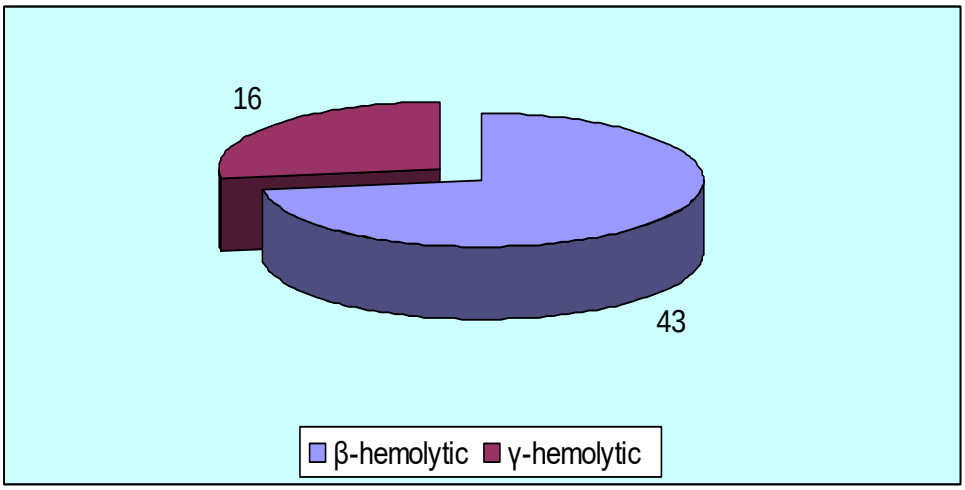

Figure (3-1) bacterial hemolysis.

3-1-3- Microscopically analysis:

The G-ve and G+ve bacteria were stained differently in this experiment. The bacteria that were chosen for this experiment were β-hemolytic when cultured on blood agar. Figure (3-2) shows that out of 43(86%), 9(21%) were G+ve and 34(79%) were G-ve.

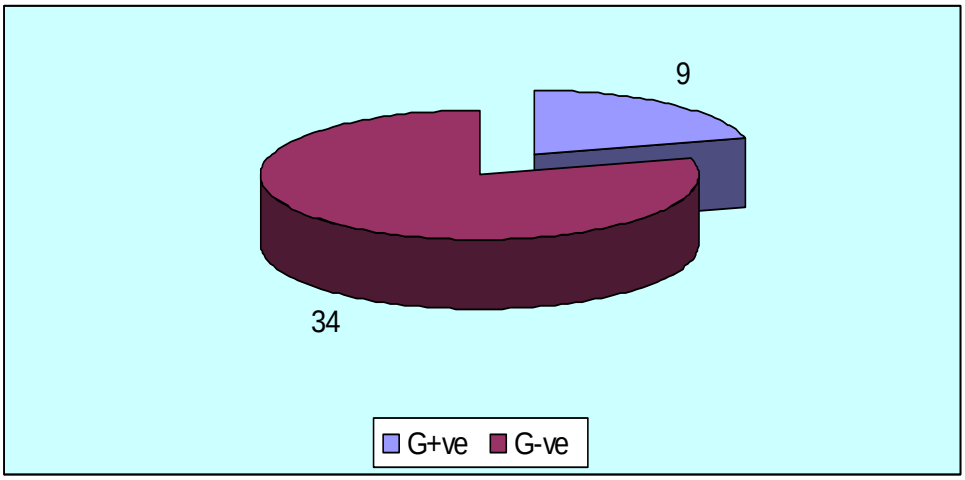

Figure (3-2) Gram stain.

The following Table (3-2) shows the morphology and aggregation of the G+ve bacteria.

Table (3-2) G+ve bacteria.

NO.	SHAPE	AGGREGATION	SPORELATION
1	cocci	Dipl-cocci	-
2	Short bacilli	Streptococcus	-
3	bacilli	Diplo-bacillus	+
4	cocci	Staphylococcus	-
5	cocci	Staphylococcus	-
6	cocci	Streptococcus	-
7	bacilli	Diplo-bacillus	-
8	cocci	staphylococcus	-
9	cocci	Staphylococcus	-

3.1.2 The sensitivity of G+ve bacteria to the plant extract(peppermint) :

Using fresh peppermint, the bacteria in this experiment were shown to be sensitive to a peppermint extract diluted 10-2. The inhibitor zone diameter was measured after adding the extract to the wells using the well diffusion method, as shown in table (3-3) for the experiment.

Table (3-3) Inhibitor zone diameter of fresh peppermint.

NO	inhibitor zone diameter(cm)		
	Watery extract	Alcoholic extract	peppermint oil
1	3.2	0	2
2	0	0	0
3	2.5	3.5	1.7
4	0	0	0
5	2.3	3.2	2.5
6	0	1	1
7	2.5	2.2	2.5
8	0	1	1
9	0	0	0

But the dehydration peppermint were the results as the following table (3-4).

Table (3-4) the dehydration peppermint.

NO	inhibitor zone diameter(cm)		
	Watery extract	Alcoholic extract	Peppermint oil
1	1.3	5	2
2	0	5	0
3	1	1.5	1.7
4	0	0	0
5	0	0	2.5
6	0	0	1
7	0	0	2.5
8	0	0	1
9	0	0	0

3-2 Discussion:

Antimicrobial activity was lower in the methanolic extract than in the aqueous extract (resuspension). This can be because the extract has poor diffusion characteristics in the agar, or it could be because the solvent has an effect on the active compounds found in fresh plants. These findings are in agreement with those of Iwalokun et al. (2004), who reported the antimicrobial effects of aqueous peppermint extract (AGE) against 133 gram-positive and gram-negative bacterial isolates, including 10 species of Candida, as well as Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Streptococcus pyogenes, Haemophilus influenzae, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli, Shigella spp., Proteus spp., and Staphylococcus aureus. The antibacterial properties of AGE were assessed using the well-diffusion and macrobroth dilution methods. The inhibition zones for gram-positive and gram-negative bacteria, respectively, were 20.2Ô22.7 mm and 19.8Ð24.5 mm, with minimum inhibitory concentration (MIC) values of 15.6Ð48.3 mg/mL and 22.9Ð37.2 mg/mL, respectively. The difference in the minimum inhibitory concentration (MIC) values at 24 and 48 hours was not statistically significant (P.05) in any of the isolates except P. aeruginosa. With no significant difference (P..05) in MIC values at 24 and 48 hours, respectively, the growth inhibition zone of 27.4 6 3.7 mm was produced by the anticandidal impact of AGE. At these incubation times, the minimum fungicidal concentrations were determined to be 14.9 and 15.5 mg/mL, respectively. Additional research showed that the antibacterial effectiveness of AGE varied with dose and duration, resulting in five different time-kill patterns for the investigated isolates. This study lends credence to peppermint's usage in Nigerian herbal treatments and health goods.

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