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Antimicrobial Effect of some Plants and Herbs

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ABSTRACT:*This study was conducted to determine the antimicrobial activity of some herbs and plants (rosemary, sage, marjoram, chicory, reeds grass) in which mixtures of their oil forms were used at different concentrations (0, 0.4, 0.8, 1.2 and 1.6 g / L) in liquid media to measure the inhibition growth of certain microbial pathogens. Each herb and plant antimicrobial inhibitory effect with the pathogens was first determined and the effective concentrations from each herb were obtained in an oil form mixture. Herb and plant oil mixture concentrations (1.2 % and 1.6 %) recorded a complete inhibition percentage (100.0 %) with all tested microorganisms, except with Aspergillus niger and Candida albicans. The herb and plant oil mixture was then added to meat products (sausages). The quality of the sausages in terms of the chemical composition of fresh and freezing storage at –18 ^{o}C for 6 months were then examined. In addition, phenolic compounds were also determined in the tested herbs and plants.*

KEY WORDS: *antimicrobial, plant oil, sausages, phonolic, pathogen.*

I. INTRODUCTION

Herbs and spices were recognized by Egyptians over 3000 years ago as preservative agents. Many types of herbs and spices are used in Egypt mainly as seasonings to improve the flavor of food, as a preservative, and as treatments for certain disease conditions. Herbs are usually only parts of plants and may be; roots, rhizomes, barks, seeds fruits, flower buds, etc. Herbs are very aromatic and may contain large percentages of essential oil as well as other powerful nonvolatile flavoring components [1].

Safe food is essential for the protection of human heath since microbial or chemical contamination of food lead to the spread of several human diseases. These kinds of contaminants find their way into food through one or more stages of food production like harvesting, processing, packaging, storage and even marketing. Microbial contamination of food represent one of the most dangerous factors which affect directly both human health and food quality due to the ability of microorganisms to produce one or more of their metabolic byproducts which have toxigenic action on human [2]. Sausage is a food that is prepared from comminuted and seasoned meat. Meat forms a main component of sausage and is a suitable medium for the growth of microorganisms. The bacteriological load of meat products depends upon the microbial load of the raw meat used for grinding, sanitary conditions, and time and temperature of storage. Other sources of microorganisms in sausages include spices, condiments, salt and natural casings. The latter have been found to contain high numbers of bacteria [3]. In most cases, the levels of spices used in the production of sausages are insufficient for their antimicrobial activity to interfere with the growth of food-borne pathogens, and hence they are not very effective as preservatives. This is in contrast with fresh meat products, where a mixture of spices can be successfully applied to stabilize the sensory appearance and hence extend the shelf life of the food. Some spices (garlic, nutmeg, mace, paprika, rosemary, and sage) contain powerful antioxidants that can extend the shelf life of sausages [4].

Staph. aureus, *Bacillus cereus*, *Clostridium botulinum*, *Streptococcus faecoles*, *Vibrio cholera*, *Salmonella sp.* and *Shigella sp.* are the major groups of bacteria which affect on the human health. On the other hand, moulds dangerously effect human health through their contamination of food due to the production of toxins which known as mycotoxins. Members of *Aspergillus*, *Penicilliurn*, *Fusarium*, *Alternaria* and *Cladosporium* are the major groups of food contamination due to their production of toxins which has the ability to withstand heat treatment during the food processing [5].The problem of food preservation has grown to be more complex as new food products are frequently being introduced on the market, requiring longer shelf life and greater assurance of protection from microbial spoilage. There are several chemicals that can be used as antimicrobial agents. For instance, acetic acid and sulfur dioxide are widely used as food preservatives. However, these chemicals require caution in handling since they are corrosive and their vapors can irritate the eyes and respiratory tract. It has been reported that sodium nitrite combines with secondary and tertiary amines, forming nitrosoamines which are carcinogenic. The most commonly used antioxidants, B H A and B H T, are added to a wide variety of foods to prevent rancidity of lipid-containing products and also have antimicrobial activity. These synthetic chemicals convert some ingested materials into toxic substances or carcinogens by the

increase of microsomal enzymes. Consequently, alternative preservatives reare needed which possess antimicrobial activity but cause no health problems to the handler and consumer [6]. Many herbs and spices display antimicrobial activities in which such antiseptic potential resides in the essential oils. In this respect, this study examined various spice essential oils for their inhibitory activity towards the growth of some micro-organisms in sausages.

II. Materials and Methods

2.1 Source of herbs

Commercially dried ground spices and its oils such as {Rosemary (*Rosemarinus officiais*), Chicory (*Cichorium intybus*), Marjoram (*Origanum marjorana*, L.), Sage (*Salvia officialis*) and Reeds grass (*Arundo domaxl*)} were obtained from local market in 2007 from Minufiya Governorate.

2.2 Microbiological cultures

Bacterial, fungal and yeasts cultures used in this study involved: *Escherichia coli* (DSM 30083), *Staphylococcus aureus* (DSM 1104), *Bacillus cereus* (DSM 315), *Salmonela* sp. (DSM 347) were obtained from Microbiological Resource Center "MIRCIN", Faculty of Agriculture, Ain Shams University, Cairo, Egypt. And mold (*Aspergillus niger*) & yeast (*Candida albicans*) were obtained from Department of Microbiology, Faculty of Science, Ain Shams University, Cairo, Egypt.

2.3 Antimicrobial activity Measurements

The antimicrobial activity was measured by the number of colonies detected on the plates. Plates were inoculated with an initial number of 1.0 X 10^6 colony forming units / gram (cfu/g) of each microbial species grown on their selective media. After which the percentage of inhibition was calculated with the decrease in microbial counts after inoculation with 1ml of the tested plant or herb samples for 22-48h at 35°C. Molds and yeast were incubated at 25°C for 7-10 days after which colonies were counted and expressed as cfu/g of sample.

2.4 Preparation of sausages samples for microbiological analysis

Meat and natural mutton casings were obtained from the local market, Minufiya Governorate.Ten grams of each sample were homogenized with 90 ml distilled water so as to give 0.1dilution. Then different dilutions ($1 : 10^{-1}$ to $1 : 10^{-6}$) were prepared to be used for microorganisms tests.

2.5 Cultivation media

Selective media for each microorganism growth was prepared into plates to which microbes were inoculated, then colonies were counted based on certain parameters. Mold (*Aspergillus niger*) and yeast (*Candida albicans*) were grown using malt – yeast extract agar and colonies were counted and expressed as cfu/g sample. *Staphylococcus aureus* were grown on Baird-Parker agar (Oxoid) and colonies were selected based on International Commission of Microbiological Specifications for Foods [7]. *Bacillus cereus* was grown using Bacillus cereus selective agar medium with supplement SR99 and colonies were selected as described by Roberts [8]. *Salmonella* sp. was grown using Salmonella sp (SS agar modified Oxoid) and colonies were selected as described by Bryan [9]. *Escherichia coli* was grown using m-Endo agar (Millipore) and colonies were selected as described by WHO [10].

2.6 Preparation of natural mutton casings

Casings were removed carefully from the slaughtered animal without punctures to avoid contamination of the carcass as well as to insure that it will be not less than minimum possible length. Three essential operations were performed prior to curing: fat and mesentery were removed as completely as possible, the intestinal contents were slipped out under a spray of water to keep the exterior clean, then slim were removed by crushing intestines manually between two successive rollers. Next, natural casings were salt cured, and were packed in barrels with salt. Prior to use, casings were soaked and washed well with water. The casings were kept wet at all times once the salt was removed from prior to filling according to the method described by El-Deep [11].

2.7 Preparation of sausages

Sausages were prepared using the following formula: Lean meat (beef) 69.05 %, Fat tissues (12.44 %), Salt(Sodium chloride) 2.225 %, Water (as ice) 15.00 %, Species mixture 0.80 %, Sodium alginate 0.50 %, Sodium nitrite 0.005 %. Imported frozen beef was thawed at room temperature and minced. The ingredients were mixed and emulsified using laboratory emulsifier (Hobart kneading machine) for sausages for 8-10 minutes. Then the emulsion was stuffed by hand into natural mutton casings (specially prepared sheep casings, diameter 80 mm).

The tested plants and herbs oil with specific concentrations were added to the sausages to determined the difference in inhibition of tested microorganisms in sausages samples. Sausages in mutton casings was stored at -18 °C for 6 months. Spoilages were detected by the development of off odors.

2.8 Analytical Methods

Moisture, Protein (N x 6.25 Keldahl method), fat (hexane solvent, Soxhielt apparatus), fiber and ash were determined according to the method recommended by A. 0. A. C. [12].

2.9 Carbohydrates and energy value

Carbohydrate was calculated by differences as follow:

% Carbohydrates = 100 - (% moisture + % protein + % fat + % ash + % fiber).

Energy value was estimated by multiplying protein and carbohydrates by 4.0 and fat by 9.0.

2.10 Evaluation of nutritional value

To evaluate different products, consumed amount of sausages to cover the daily requirements of adult man (G. D. R. g) in protein or energy (63 gm and 2900 kcal., respectively and calculated according to RDA [13]. Percent satisfaction of the daily requirements of adult man in protein and energy when consuming 150 mg of sausages (P. S. /150) was also calculated.

2.11 Determination of thiobarbituric acid value (T. B. A.)

Ten grams of sample was distilled (distilled water + 4N Hcl) for 10 minutes, 5 ml. of the distillate was added to 5 ml. T. B. A. solution (0.28839g T. B. A. / 100 ml of 90% glacial acetic acid) into a stoppered tube, which was then heated in boiling water for 35 minutes. After cooling measurements were carried out calorimetrically at 538 nm., the T.B.A. value was calculated by multiplying the absorbency by the factor (7.8) and the results were presented as grams of malonic / kg sample.

2.12 Extraction and identification of phenolic compounds

A known weight of dried powdered sample was soaked in 25 ml sterilized water and agitated on a rotary shaker for 24 h. at 200 rpm. Sullary was filtered through Whatman 3 MM filter paper under vacuum, followed by centrifugation at 12,500g for 30 min. at 80 °C. The aqueous extract was acidified to 2.5 pH using diluted phosphoric acid. Each sample was partitioned three times with an equal volume of diethyl ether. The combined diethyl ether layers was evaporated to dryness under reduced pressure at 30 °C. The resulting residue was re- dissolved in 3 ml of spectral grade methanol and filtrated through a 0.2 µm filter sterilized membrane prior to HPLC analysis. Phenolic compounds of herb and plant samples were extracted according to the method outlined by Ben-Hammoudam [14]. The standards of phenolic compounds were purchased from Sigma (St. Louis, USA) and from Merck-Schuchardt (Munich, Germany) chemical companies.

III. RESULTS AND DISCUSSION

3.1 Antimicrobial Activity

The inhibitory effect of each plant species (rosemary, sage, marjoram, chicory and Reeds grass) under various concentrations (0.4 %, 0.8 %, 1.2 % and 1.6) were investigated on some pathogenic bacteria and fungi species enumerated in liquid media. Data presented in Fig. (1) shows a complete inhibition (100.0%) of *E. coli*, *Salmonella sp, Bacillus cereus* and *Staphylococcus aureus* with rosemary oil concentration of 1.6%. In addition, this concentration gave a maximum inhibition percentage of 99.98 and 99.99 % with fungi species *Aspergillus niger* and *Candida albicans* respectively. In Fig.2, there is a complete inhibition (100.0%) of *E. coli* and *Salmonella sp.* was recorded with all tested sage oil concentrations (0.4 %, 0.8 %, 1.2 % and 1.6 %). A complete inhibition (100.0%) of *Bacillus cereus* was recorded with 1.2 % and 1.6 % sage oil concentrations. The maximum inhibition percentage of *Staphylococcus aureus* was recorded with 1.2 % and 1.6 % sage oil being 99.999 % and 99.999 %, respectively. A markedly reduction of *Aspergillus niger* and *Candida albicans* was observed especially with 1.6 % sage oil concentration being 99.99 % and 100.0 %, respectively. Data in Fig. 3 shows the maximum value of inhibition percentage of 0.4 % and 0.8 % marjoram oil concentrations was recorded with *Candida albicans* and *Staphylococcus aureus*. The values were 99.95 % and 99.98 %, respectively while the lowest inhibition percentage was recorder with *Bacillus cereus (*96.50 % and 97.00 %). Marjoram oil concentrations (1.2 % and 1.6 %) recorded the maximum value of inhibition percentage with *Staphylococcus aureus* and *Candida albicans*. The values were 99.998 %, 99.999 %, respectively. While the lowest inhibition percentage was recorder with *Bacillus cereus* (99.40 % and 99.85 %).In Fig. 4, the highest value of inhibition percentage of 0.4 % and 0.8 % chicory oil concentrations was recorded with *Staphylococcus aureus*.

The values were 99.98 % and 99.99 %, respectively. However, the lowest inhibition percentage was recorder with *Candida albicans* (99.70 % and 99.80 %). Chicory oil concentrations (1.2 % and 1.6 %) recorded the highest value of inhibition % with *E. coli*. The values were 99.99 %, 99.999 %,respectively. While the lowest inhibition percentage was recorder with *Candida albicans.* The values were 99.85 % and 99.98 %. Data in Fig. 5 presents the maximum value of inhibition percentage of 0.4 % and 0.8 % reeds grass oil concentrations was recorded with *Staphylococcus aureus.* The values were 99.95 % and 99.98 %, respectively. While the lowest inhibition percentage was recorder with *E. coli* (99.98 %and 99.99%). Reeds grass oil concentrations (1.2 % and 1.6 %) the maximum value of inhibition percentage was recorded with *Candida albicans*. being 99.998 %, 99.999 %, respectively. The lowest inhibition percentage was recorder with *E. coli* and *Salmonella sp.* (99.83 % and 99.94 %). Data in Fig.6 shows the highest value of inhibition percentage of 0.4 % and 0.8 % herb and plant mixtures oil concentrations was recorded with *Staphylococcus aureus*. The values were 99.999 % and 99.999 %, respectively. While the lowest inhibition percentage was recorder with *E. coli* and *Aspergillus niger* (99.80 % and 99.93 %). Herb and plant oil mixture concentrations (1.2 % and 1.6 %) recorded a complete inhibition percentage (100.0 %) with all tested microorganisms, except with *Aspergillus niger* and *Candida albicans.*

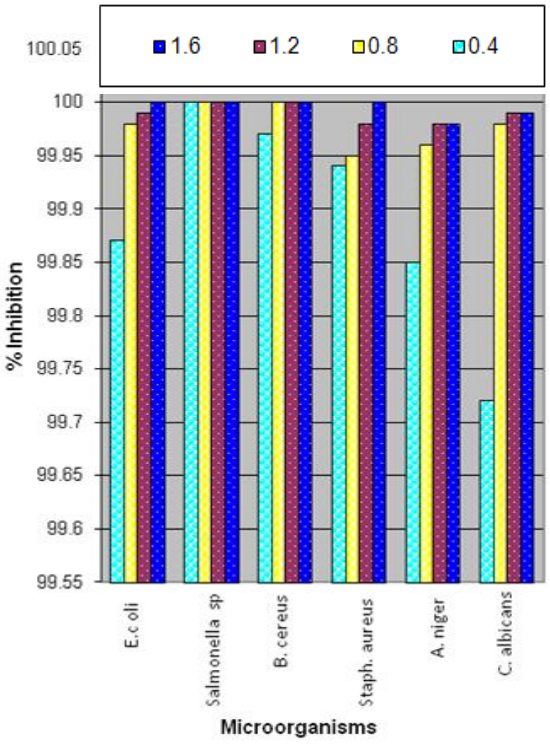

Figure 1. The Inhibitory effect of various rosemary oil concentrations (0.4, 0.8, 1.2, 1.6) on pathogenic bacteria (*E. coli, Salmonella sp., Bacillus cereus, Staphylococcus aureus*) and fungi (*Aspergillus niger* and *Candida albicans*).

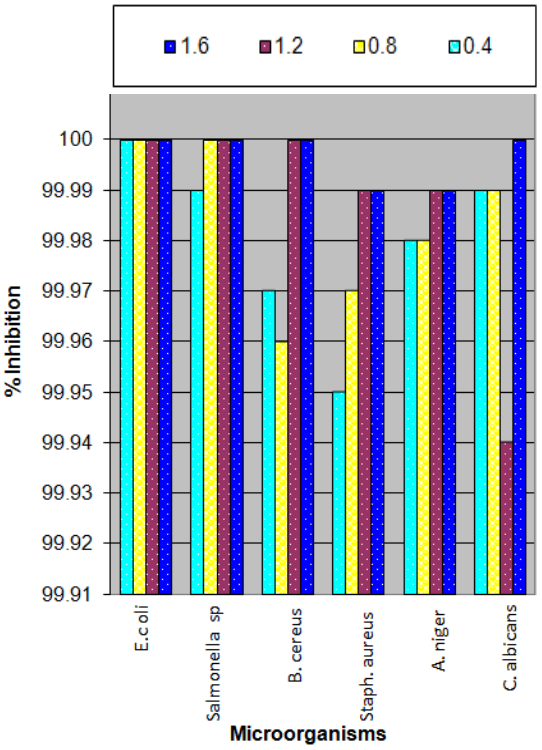

Figure 2. Inhibitory effect of various sage oil concentrations (0.4, 0.8, 1.2, 1.6) on pathogenic bacteria (*E. coli, Salmonella sp., Bacillus cereus, Staphylococcus aureus*) and fungi (*Aspergillus niger* and *Candida albicans*).

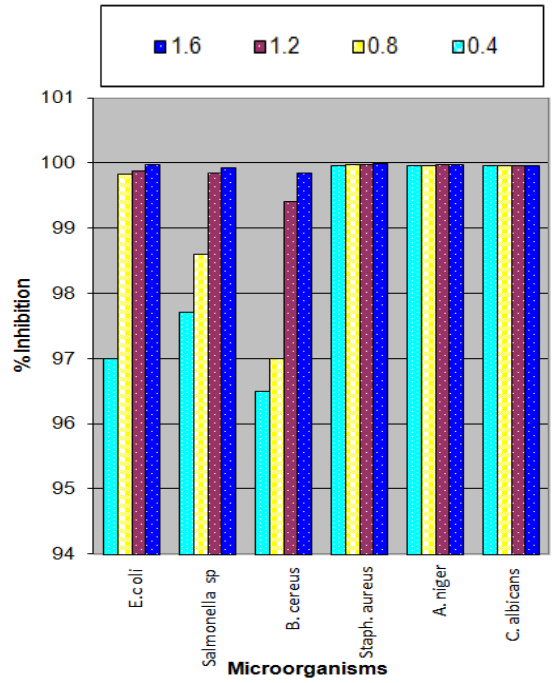

Figure 3. Inhibitory effect of various marjoram oil concentrations (0.4, 0.8, 1.2, 1.6) on pathogenic bacteria (*E. coli, Salmonella sp., Bacillus cereus, Staphylococcus aureus*) and fungi (*Aspergillus niger* and *Candida albicans*).

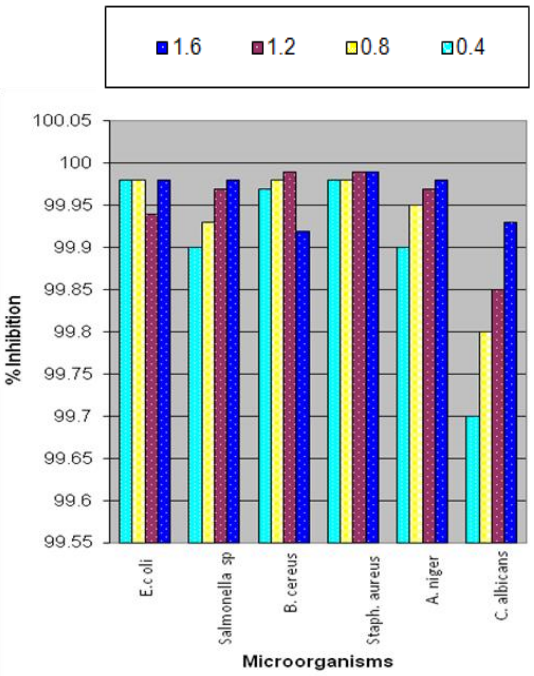

Figure 4. Inhibitory effect of various chicory oil concentrations (0.4, 0.8, 1.2, 1.6) on pathogenic bacteria (*E. coli, Salmonella sp., Bacillus cereus, Staphylococcus aureus*) and fungi (*Aspergillus niger* and *Candida albicans*).

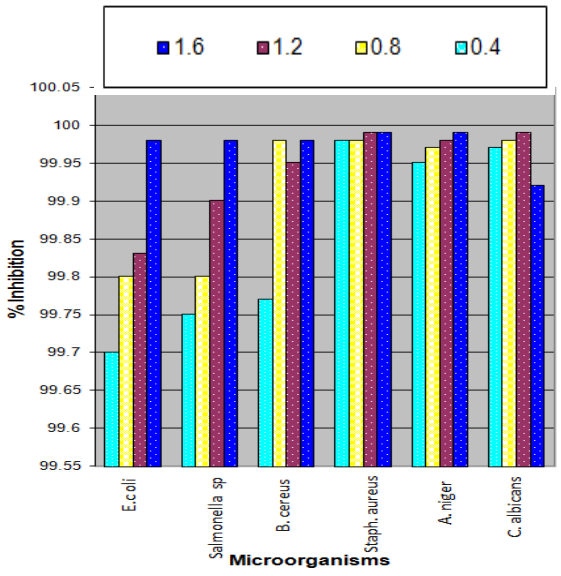

Figure 5. Inhibitory effect of various Reeds grass oil concentrations (0.4, 0.8, 1.2, 1.6) on pathogenic bacteria (*E. coli, Salmonella sp., Bacillus cereus, Staphylococcus aureus*) and fungi (*Aspergillus niger* and *Candida albicans*).

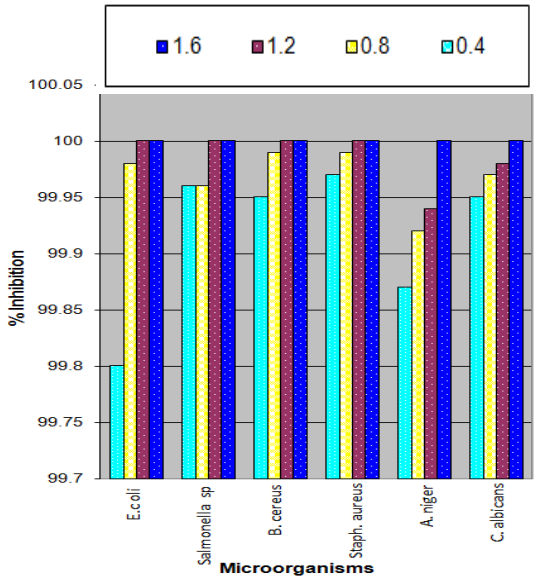

Figure 6. Inhibitory effect of herbs and plants mixture oils at various concentrations (0.4, 0.8, 1.2, 1.6) on pathogenic bacteria (*E. coli, Salmonella sp., Bacillus cereus, Staphylococcus aureus*) and fungi (*Aspergillus niger* and *Candida albicans*).

3.2 Sausage quality

3.2.1 Chemical composition of fresh sausages

Data presented in Table 1, shows the chemical composition of fresh sausages as influenced by the addition of the best selected microbial inhibitory concentration for each herb and plant oil mixed together (on wet weight basis). Control sausage (without herb and plant mixtures) recorded the highest moisture, protein, and ash, content (%) as wet weight. Sausage with herb and plant mixture oils recorded the highest fat content percentage(19.37%) as well as carbohydrates (3.43%) and energy (245.23 kcal/100g), while the lowest values recorded with protein (14.33%) and ash (1.66%) content. In addition, control sausage recorded the highest GDR for energy (1209 g) and PS/150 (35%) for protein. In contrast, sausage with herbs mixture oils recorded the highest PS/150 (12.67%) for energy and GDR (440) for protein.

3.2.2 Chemical composition of sausages as influenced by addition of herb and plant mixtures oils during frozen storage at -18°C for 6 months

Data presented in Table 2, shows the chemical composition of sausages stored at -18 °C for 6 months and the influence of the addition of the best selected microbial inhibitory concentration for each herb and plant oil mixtures (on wet weight basis). The control sausage (on wet weight basis) recorded the highest values of moisture, ash, fiber, GDR for energy and PS/150 for protein. However, the lowest values for protein (12.17) and fiber (0.10) content were recorded for sausage with herbs and plants mixture oils. PS/150 for energy(13.76), showed similar values for control sausage and sausage containing herb and plant mixture oils. With progress of frozen storage period, sausages containing herbs and plants mixtures oils showed a decrease in the moisture (58.36%), ash(1.96), protein(12.17), fiber (0.10), GDR for energy(1090) and PS/150 for protein(29) decreased. While the fat(21.56), carbohydrates (5.85), energy value(266.12), GDR for protein (518) and PS/150 for energy(13.76) increased. Fat and water are the two major components influencing the quality, yield and stability of sausages [15]. Lean meat is the most important ingredient of sausages as water plays a role in binding, mantaning the fat component of the mixture and in determining product cohesiveness. Thus, if the lean meat contant was high the quality of the end products will also increase. As the percentage of fat in sausage increase the moisture content decreases. Fat contributes greatly to the platability of sausages, it serves as the discontinuous phase of sausage emulsions. Water plays a role in platability and in increasing meat binding and providing fluid conditions during fat chopping. The most important factor that affects water content of sausage is the moisture [16]. Reagan et al. [17] found that sausage samples with lower fat levels (30.3%) had a higher content of lean mean and a higher content of moisture (51.8%). These results are in agreement with our results since both the water and fat content of sausages effect platability as they increase the tenderness and juiciness.

Table 1. Chemical composition of fresh sausage as influenced by addition of herbs and plants mixture oils.

Constituents	Moisture %	Protein %	Fat %	Ash %	Fiber %	Carbohydrates %	Energy value	GDR Forprotein (g)	GDR for energy (g)
Sausage (control)	61.6	14.7	18.9	1.7	0.25	2.6	239.8	426	1209
Sausage + herbs mixture oils	61.2	14.3	19.3	1.6	0.03	3.3	245.1	440	1183

Table 2. Chemical composition of sausage as influenced by addition of herbs and plants mixture oils during frozen storage at -18 °C for 6 months.

Constituents	Moisture %	Protein %	Fat %	Ash %	Fiber %	Carbohydrates %	Energy value	GDR Forprotein (g)	GDR for energy (g)
Sausage (control)	58.8	12.4	21.0	2.1	0.31	5.2	260.3	504	1114
Sausage + herbs mixture oils	58.3	12.1	21.5	1.9	0.10	5.8	266.1	518	1090

3.3 Microbiological aspects of fresh sausages as influenced by addition of herb and plant mixtures oils during frozen storage at -18 °C for 6 months

Data given in Fig.7, shows the inhibitory effects of herbs and plants mixtures oil on some pathogenic microorganisms enumerated in fresh sausages during storage period at – 18 °C for 6 months. At zero time the counts of all tested microorganisms(*E. coli, Salmonella sp., Bacillus c*) *ereus, Staphylococcus aureus, Aspergillus niger* and *Candida albicans* were 1.0 x 10^6 cfu / g in sausage sample containing the best selected microbial inhibitory concentrations fir each herb and plant oil mixture. At the end of storage period of 6 months at – 18 °C the counts of all tested microorganisms in sausage samples recorded the highest inhibition by various rates. The counts were 5.0 x 10^3, 2.0 x 10^3, 1.5 x 10^2, 0.4 x 10^3, 1.5 x 10^2 and 2.1 x 10^2 cfu / g for *E. coli, Salmonella sp., Bacillus cereus, Staphylococcus aureus, Aspergillus niger* and *Candida albicans*, respectively; showing percent decrease of 99.5 – 99.99 %. Therefore, these results indicate that both the effect of lower temperature and addition of herbs and plants mixture oils significantly allows for preservation of sausage samples for longer periods without the need to use hazardous preservatives.

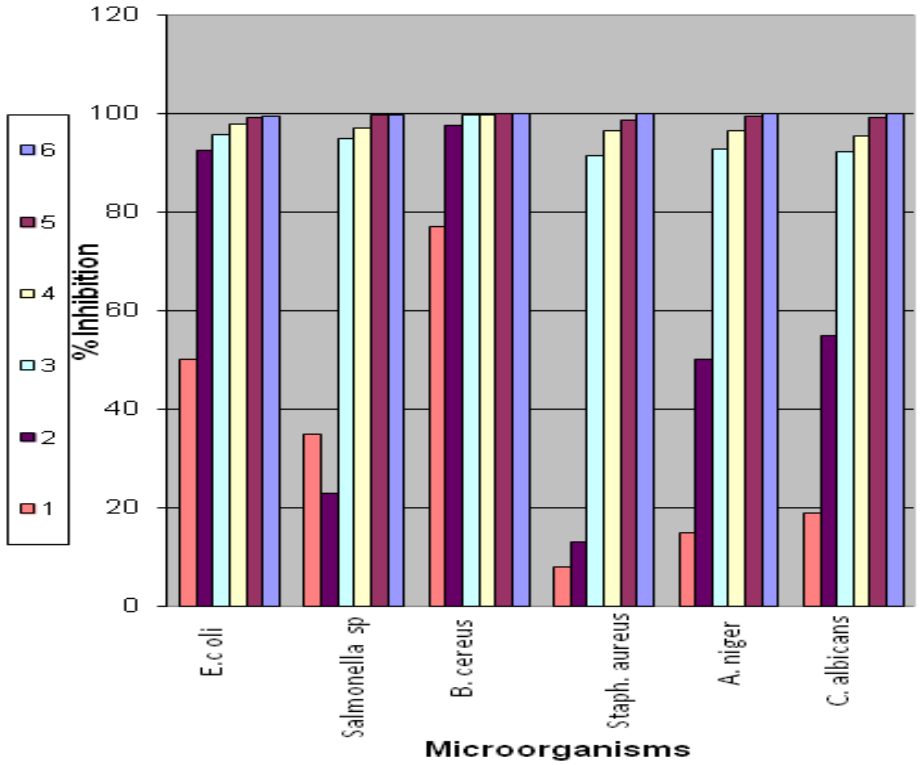

Figure 7. Inhibitory effect of herbs and plants mixture oils on pathogenic bacteria (*E. coli, Salmonella sp., Bacillus cereus, Staphylococcus aureus*) and fungi (*Aspergillus niger* and *Candida albicans*) inserted in sausage during storage period for 6 months at -18 °C .

3.4 The effect of herbs and plants mixtures oil on thiobituric acid values during frozen storage for 6 months

The changes in thiobituric acid (TBA) value of sausages as influenced by the addition of herbs and plants mixtures oil during frozen storage period at – 18 °C for 6 months is shown in Table 3. At zero time of storage period at– 18 °C the values of T.B.A. were 0.15 and 0.23 mg / kg for control sausage and sausage with herbs and plants mixture oils, respectively. At the end of frozen storage (6 months) the TBA values for the control increased to 1.54 while for the sausage samples containing herbs and plants mixture oils the TBA value decreased to 1.30 and lower than the control. The 2-thiobituric acid (TBA) test has been widely used for measuring oxidative rancidity in fat-containing food, especially in meat products. A reduction of TBA reactive substances at freezer temperatures was found to occur with advanced lipid oxidation [18-20]. Processed meat such as fresh sausage, were oxidation prone in frozen storage conditions, in which griding greatly increases the exposure of lipids to air. In addition, the heme pigments in meat are strong oxidation catalysts when brought into contact with lipids [21]. However, previous studies have shown that the use of antioxidants in reconstructed beef steaks controlled lipid oxidation at significantly lower levels than that of the controls without any

antioxidants added. Results of this study indicate that the reduction in T.B.A values is due to higher antioxidation activity of herbs and plants mixture oils during long frozen storage periods.

Table 3. Changes in thiobarbituric acid value of sausages as influenced by addition of herbs oil mixtures during frozen storage at -18 °C for 6 months (mg /Kg).

Storage Period (months)	Sausage (control)	Sausage +herbs mixture oils
Zero time (0)	0.32	0.15
1	0.52	0.42
2	0.69	0.51
3	0.85	0.50
4	1.25	1.15
5	1.40	1.25
6	1.54	1.30

Table 4. Determination and identification of phenolic compounds in tested herbs and plants.

Phenolic compounds standards	Percentage of Phenolic Compounds of Tested Herbs and Plants (%)				
	Rosemary	Sage	Marjoram	Chicory	Reeds grass
Pyrogallic	3.96	1.57	4.74	1.09	0.96
Coumarin	ND	ND	5.55	ND	ND
Protocatechuic	0.22	ND	ND	0.06	ND
P.Oh benzoic	ND	ND	0.68	0.14	1.20
Cinnamic	0.23	ND	ND	ND	ND
Catechin	ND	0.73	ND	ND	ND
Quercetin	ND	ND	ND	0.022	0.07
Gallic	0.20	1.63	0.08	ND	0.33
Salicylic	ND	ND	ND	ND	ND
O-Coumaric	0.48	0.58	0.72	0.076	ND
Phenol	0.81	1.43	ND	0.12	0.08

ND- Not Detected

3.5 Determination and identification of phenolic compounds in tested herbs and plants

Results in Table 4 demonstrates the presence of eleven different phenolic compounds was investigated in the studied herbs and plants (rosemary, sage, marjoram, chicory and Reeds grass). All testes herbs and plants contained different concentration of phenolic compounds. Rosemary herb contained some of phenolic compounds, such as Pyrogallic, protocatechuic, cinnamic, gallic and phenol. Sage contained many phenolic compounds such as pyrogallic, catechin, gallic, O-Coumaric and phenol. Marjoram herb, contained many phenolic compounds such as pyrogallic, coumarin, P.Oh benzoic**,** gallic and O-Coumaric .Chicory plant using HPLC showed some phenolic compounds such pyrogallic, protocatechuic, P.Oh benzoic, quercetin**,** O-

Coumaric and phenols. Reeds grass contained some of phenolic compounds such as pyrogallic, P.Oh benzoic**,** quercetin, gallic and phenol . Overall, all tested herbs and plants were mixed to give the best results for inhibition of tested microorganisms and to improvement the sausage quality.

IV. CONCLUSION

The growing concern about safety of foods has recently led to the development of natural antimicrobials to control foodborne pathogens. Herbs are some of the most commonly used natural antimicrobial agents in foods. Addition of spices in foods not only imparts flavor and pungent stimuli but also provides antimicrobial property. Extracts of many aromatic plants and herbs had the ability to retard the microbial invasion into the food in addition to import attractive flavor and aroma herbs extracts are more preferred rather than synthetic preservatives because it has no side effect when used at proper level and not accumulated in most of synthetic preservatives. So, application of proper practices during each stages of the production of food could improve the quality of it. In most cases, the levels of spices and herbs used in the production of sausages are insufficient for their antimicrobial activity to interfere with the growth of food-borne pathogens, and hence they are not very effective as preservatives. This is in contrast with fresh meat products, where a mixture of spices can be successfully applied to stabilize the sensory appearance and hence extend the shelf life of the food [4].

The antimicrobial properties of herbs have been long studied. The antiseptic potential of spices resides in the essential oils in which extensive studies have been performed to determine their inhibitory properties, and many food-borne pathogens, both gram-positive and gram-negative bacteria, have been shown to be inhibited by spices [22]. On the other hand , many of aromatic plants and herbs like chili, mint, clove, dill, parsley, cumin, coriander and others are used in our daily dishes to give highly acceptable seasoning properties for the foods. Recent studies indicated that beside the seasoning properties of many seasons and herbs they have also clear and pronounced antimicrobial activities against many of bacteria and moulds that related to food spoilage. Thus, the new international trend of food specialists is directed into the use of these natural herbs and plants and their extracts as the food preservatives. Indeed, the oxidation of lipids in foodstuffs results in the development of off flavors, resulting in a product that is unacceptable for human consumption [23]. The fat content in frozen sausages will lead to oxidative rancidity due to excessive long storage periods. Even though the percentage of fat increase in sausages containing herbs and plants mixtures oils during long periods frozen conditions, the TBA value didn't increase but rather decreased. This shows the ability of these tested herbs and plants to act as antioxidants and decrease lipid oxidation in addition to their antimicrobial properties. The presence of various phenolic compounds in different concentrations also allowed for the increase in shelf life of sausages. This study has shown that the mixture of some herbs and plants (rosemary, sage, marjoram, chicory and reeds grass) with their best selected concentration (1.2 % and 1.6 %) gave the highest antimicrobial activity against major pathogenic microorganisms (*E. coli, Salmonella sp., Bacillus cereus, Staphylococcus aureus, Aspergillus niger* and *Candida albicans*). Furthermore, these herbs and plants mixtures have demonstrated antioxidant properties that lead to increase the shelf life of sausages and thus are a great natural source of preservatives.

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