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Effect of Vacuum Packaging and Natural Ingredients on the Physical and Microbiological Properties of Fresh Oregano (*Origanum syriacum*) Products

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Abstract Fresh oregano (Origanum syriacum L.) is considered one of the most commonly used aromatic herbs in the Mediterranean diet. This study aimed to evaluate the possibility to extend the shelf life of fresh oregano leaves by employing vacuum packaging and natural ingredients. In this study, 132 samples of fresh oregano have been prepared in vacuum packs and divided into four treatments (n=33/treatments). The oregano recipes treatments were labeled as A (Only fresh oregano leaves 100%, Control), B (fresh oregano 63.2%, fresh onion 15%, olive oil 20%, NaCl 1.8%), C (fresh oregano 61.91%, fresh onion 15%, olive oil 20%, NaCl 1.8%, sumac powder 1.29%), and D (Fresh oregano 59.2%, 15% Fresh onion, 20% oil, 1.8% salt, 4% lactic Acid, ultimate pH 4.4). The potential growth of *Clostridium botulinum* by using *Clostridium sporogenes DSM795* as a surrogate microbe has been assessed. Moreover, color attributes (*L**, *a**, *b**), microbiological counts (aerobic, anaerobic, and psychrotrophic as well as yeast and molds), and pH- values have been evaluated during the storage period (42 days). Both spot and spreading agar journey methods showed that groups B and D could be resisted the growth of *Clostridium sporogenes DSM 795*. It was found that lactic acid was the most effective ingredient against aerobic, anaerobic, and psychrotrophic bacteria if compared to sumac and onion. On another hand, Group C showed significantly ($p<0.05$) the lowest *L** and *b**-values if compared with other groups. In conclusion, the addition of lactic acid (group D) was the most effective antimicrobial agent in comparison with other ingredients. In addition, lactic acid enhanced the safety of the product by inhibition of the growth of *Clostridium sporogenes DSM 795*.

Keywords: *oregano, color traits, Clostridium sporogenes, vacuum, sumac*

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1. Introduction

Origanum syriacum L. (Lamiaceae) is one of the most common herbs in the Mediterranean region. In Palestine and other Middle East countries, the fresh and dried leaves of *Origanum syriacum* have wide culinary uses in bakery products such as "Kras Alzaater" (made of fresh leaves), "mankouchi" (dried milled leaves mixed with spices), etc. [1]. *It includes annual, biennial medicinal and aromatic plants. It is commonly used in the food, cosmetics, aromatherapy, and pharmaceutical industries. Origanum species (*O. vulgare, O. majorana, *and* O. syriacum) *have wide bactericidal and fungicidal effects* [2]. There are differences between species including morphological, phenological, genetic and chemical characteristics of the grown and wild species lead to the identification of Origanum in the entire world [3].

Origanum syriacum is a perennial plant. It produces white to pink flowers which have aromatic properties. Leaves are used as fresh or whole/crushed dried [3]. A long time ago, Oregano was used as a traditional medicine for different diseases. For example, it used for the treatment of respiratory disease whooping cough, bronchitis and asthma [4]. Oregano had unique bioactive components such as thymol and carvazole [5] as well as it is rich in folic acid, beta carotene, and vitamins (A, C, E, K) [6]. It was found that oregano exhibited a significant level of phenolic compounds such as zea-xanthin, pigenin, lutein, luteolin and thymonin [4,7].

Recently, it was found that essential oils of origanum had therapeutic effects against several diseases such as Alzheimer's disease. Moreover, it had an anti-inflammatory effect, antimicrobial effect (anti-bacterial, anti-oxidizing, and anti-fungus), as well as increase the antibiotics sensitivity for resistant microbes [8,9,10,11].

In the last years, the cultivation of oregano is economically becoming feasible in Palestine. According to the Palestinian Ministry of Agriculture, there is 5500 dunum produce 11000 tons of green oregano per year. The life cycle of oregano is usually durable for seven years or less [12].

Fresh oregano is a seasonal crop. To cover the off-seasons, the traditional preservation methods such as freezing, drying, and packing in plastic bottles are commonly used in Palestine and the Mediterranean region [12]. The common traditional preservation methods may be led to a loss of nutritional values and impair some of the sensory properties like color, taste, and flavor [13]. Until now, there is no commercial method available in Palestine to increase the availability of fresh oregano leaves during off-season or summer period. Therefore, the aim of this study to evaluate the possibility to extend the shelf-life of fresh oregano leaves using hurdle technology (employing vacuum packaging technique combined with natural ingradients).

2. Materials and Methods

2.1. Samples Collection and Preparation

About 5 kg of fresh oregano leaves have been harvested from a field near Tulkarem city, Palestine. The harvested area was carefully selected to be free from wild plants and any abnormalities. The leaves were manually separated from the stems. The impurities such as weeds, straw, stems, gravels, etc were manually separated. Ultimately, the oregano leaves were cleaned under running tap water until the output water got clear. The leaves were left to dry on paper towels. The whole quantities of leaves were mixed thoroughly to obtain a homogenous mixture.

2.2. Treatments

The whole quantities of leaves were split into four batches which represent four treatments as shown in the following Table 1:

Table 1. The list of ingredients and their percentages that were included in each treatment

	Group A %	Group B %	Group C %	Group D %
Fresh oregano leaves	100	63.2	61.9	62.7
Onion	0	15	15	15
Sumac	0	0	1.3	0
Oil	0	20	20	20
Salt	0	1.8	1.8	1.8
Lactic acid*	0	0	0	0.5

*The addition of lactic acid in group D resulted in ultimate pH 4.4

From each treatment, about 60 g of oregano leaves mix has been weighted in each vacuum pack and then sealed under vacuum (-0.95 bar). In each group, there were 33 packs and in total 132. The samples were stored at room temperature (25°C) for 42 days. At each week of the storage periods, microbiological tests (total aerobic bacteria, total anaerobic bacteria, *Clostridium botulinum* challenge test, pyschrotrophic bacteria, and yeast and mold) and physic-chemical properties (pH, color attributes *L**, *a**, *b**) have been measured.

2.3. Color Measurement

During the whole period of storage 6 samples have been selected from each group to evaluate the color coordinates (*L**, *a**, *b**) based on the CIE system (Commission Internationale de l'Eclairage). From each sample, 3 circular areas were highlighted to fit exactly the 8 mm measuring window of Minolta Chroma Meter (CR-410, Japan). In each area, three colour measurements have been collected and the mean values of three measurements have been calculated. Reflectance colorimeter (Minolta Chroma Meter CR-400) with Control (C) as an illuminate source was used to carry out the measurements. The colorimeter was calibrated with a reference white ceramic tile (Y = 93.9, x = 0.3130 and y = 0.3190) before measurement.

2.4. pH Measurement

Each treatment about 2.5g of fresh oregano collected and homogenized with ultra-turrax in 25 mL of distilled water to measure pH value by using a pH-meter ((ISFET, Model # IQ150, IQ Scientific Instruments, San Diego, USA).

2.5. Microbiological Analysis

In this study, a total of 12 replicates for each group were used to estimate the total count of aerobic (n=4), anaerobic (n=4), and psychrotrophic bacteria (n=4) during the whole storage period. Under the aseptic condition, 10 grams of oregano sample was weighted into a sterile stomach pack and then a 90 ml ringer solution was added. The sample was homogenized using the stomacher mixer (Lab Blender Seward, PBI International) for 2 min. Suitable serial dilutions (up to 10^{-7}) were prepared and used to count bacteria, molds and yeasts growth. Plate Count Agar (Oxoid) was used as a microbiological growth medium to assess total viable aerobic and anaerobic bacterial growth. To estimate total aerobic count, the plates were incubated at temperature 37 °C for 48-72 hr. To determine anaerobic bacterial count, the plates were incubated in an anaerobic jar at 37 °C for 48 hrs. Psychrotrophic bacteria count was estimated by incubation of plates at 6-7 °C for 10 days. Potato Dextrose Agar (Oxoid) was used to enumerate molds and yeasts. The plates were incubated at room temperature (25°C) for 4-5 days.

2.6. Microbiological Challenge Testing

Clostridium sporogenes DSM 795 (Bactiva GmbH, Germany) was used as a surrogate for *Clostridium botulinum* growth. The test was divided into two parts. In the first part, the potential growth of *Clostridium sporogenes* DSM 795 in each recipe (or group) has been evaluated by using Agar journey and sensitivity test methods.

Pour technique: 40 ml of previously prepared of Tryptic soy agar (TSA) culture was pumped into a falcon tube. After that, 0.5 ml of broth *C. sporogenes* was added and well-mixed. The mixture of TSA culture and broth of *C. sporogenes* was poured into plates. 10 µl of recipe

solution was transferred to the plates as a spot for each group. Moreover, the spreading method was used by transfer 100 µl to TSA plates. Finally, all plates were incubated under vacuum conditions at 37°C for 24 hr.

Sensitivity test method: 100 µl of broth *C. sporogenes* was added into previously prepared TSA plates then speared on the surface then the pates stored in the fridge for 30 min. After that, 10 µl and 20 µl of recipe solutions spotted on each paper disk, then transferred to TSA plates. Finally, all plates were stored under vacuum conditions at 37°C for 24 hr.

In the second part: The potential growth of *C. spergenes* in fresh oregano samples (green thyme, recopies, vacuum packaging) during 7 days storage period (0, 1, 3, 7 days) has been evaluated. 10 grams of oregano leaves were weighted. Then washed and dried well. *C. sporogenes* broth was diluted into 10^{-4}. Five ml of diluted *C. sporogenes* was sprayed on the 10 grams of green thyme leaves which previously weighed. Then, sprayed leaves were left for almost 15 min at room temperature to dry. After drying, the ingredient for each group was added. Vacuum packaging was applied for samples and stored at room temperature. 10 grams of sample was weighted in 90 ml of ringer solution; the mixture was homogenized by stomacher for 3 min at 400 p/min speed. Stock sample (10^{-1} dilution) was incubated in a water bath at 80°C for 10 min. Then, the mother sample was diluted up10^{-4}. The diluted samples were inoculated to TSA plates. Finally, the samples were incubated under vacuum condition by jars at 37°C for 24 hr.

2.7. Statistical Analysis

The effect of treatments and storage time on the quality traits (Physic-chemical, and microbiological properties) have been evaluated by ANOVA. The effect of treatments and the interaction effects (treatment X storage time) were assessed using the general linear model (GLM) through SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). Tukey's honestly multiple range test was used to separate the means using with $P \leq 0.05$ considered as significant.

3. Result and Discussion

3.1. pH Values of Resultant Treatments

The pH values of the resultant recipes during the whole storage period (42 days) were shown in Figure 1. The pH is considered one of the most important factors that affect the survival and growth of microbes during processing, storage, and distribution of food products [14]. pH-values during storage can indicate microbial growth and thus the spoilage. At the starting point (day 0), group A had the highest value of pH and group D exhibited the lowest value. Group B and C had statistically similar pH values. The main differences in pH values at the starting point may be attributed to the additives in each group. Group A exhibited the highest pH-value because there were no additives; it was contained just fresh leaves of oregano. The lowest pH value for group D can be attributed due to the addition of lactic acid. During the storage period (42 days), pH was moderately decreased in all groups and this may be attributed due to microbial growth especially lactic acid bacteria. At the end of the storage (day 42), all groups exhibited moderate changes in pH value if compared to the starting point.

It was found that the pH in all groups at room temperature was affected by three main factors: additives, storage periods, and storage conditions (vacuum packaging and temperatures). It was observed that there was a sharp drop down in pH for groups B and C in the first two weeks. The changes that occurred in pH for each treatment during the storage period were affected clearly by natural additives. The lactic acid was an effective substance among other additives in pH reduction. The lactic acid in group D led to drop down in pH to 4 which effected on microbial growth, color, and shelf-life. The addition of sumac in group C led to a reduction in pH which may be attributed to the fact that sumac is rich in phenolic acid, and quinines which led to a reduction in pH [15].

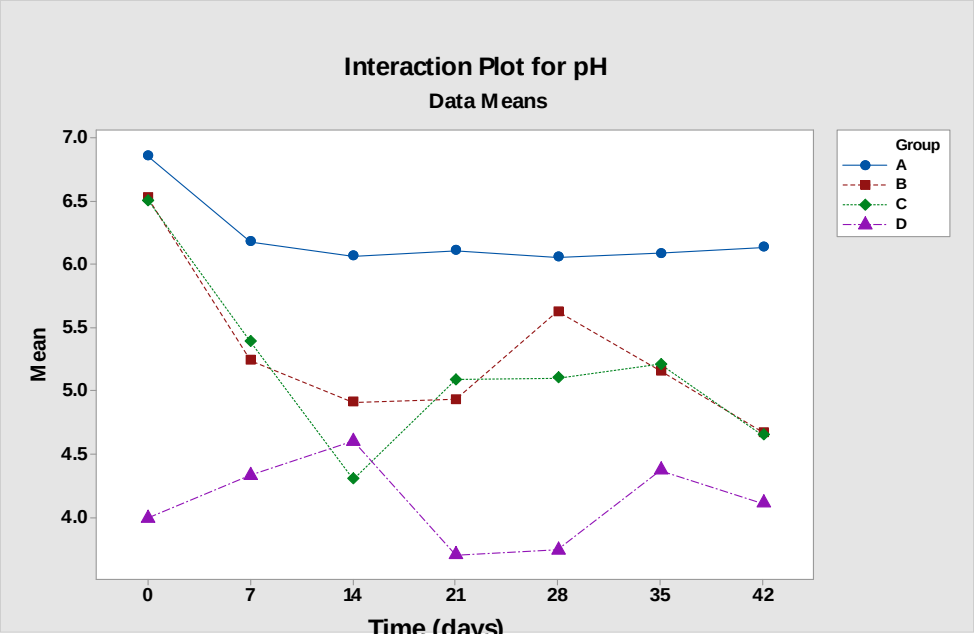

Figure 1. pH of fresh oregano samples during the storage period (42 days) at room temperature 25°C

3.2. Microbiological Status in the Resultant Recipes

The total aerobic bacterial (TAB) count was shown in (Figure 2). At the starting point (Day 0), groups A and C had no significant differences in aerobic microbial count while they were significantly higher than groups B and D. Group D exhibited the lowest aerobic count if compared with other groups. The growth patterns of aerobic bacteria during the whole period of storage were similar in groups B and C. Group D had the lowest initial aerobic bacterial count. Even the whole quantity of fresh oregano leaves which were used to prepare all treatments; was mixed thoroughly but it was difficult to obtain homogeneous initial bacterial load for all groups. These differences may be attributed to the ingredients that were added for each group.

It was clear that the addition of lactic acid was the most effective ingredient against aerobic bacterial growth as was shown in group D (pH 4.4). In the second-order, sumac exhibited a preservative effect as shown in group C. Sumac contained bioactive components (phenolic acid and quinines) which worked as an antimicrobial agent against gram-positive and negative bacteria [15].

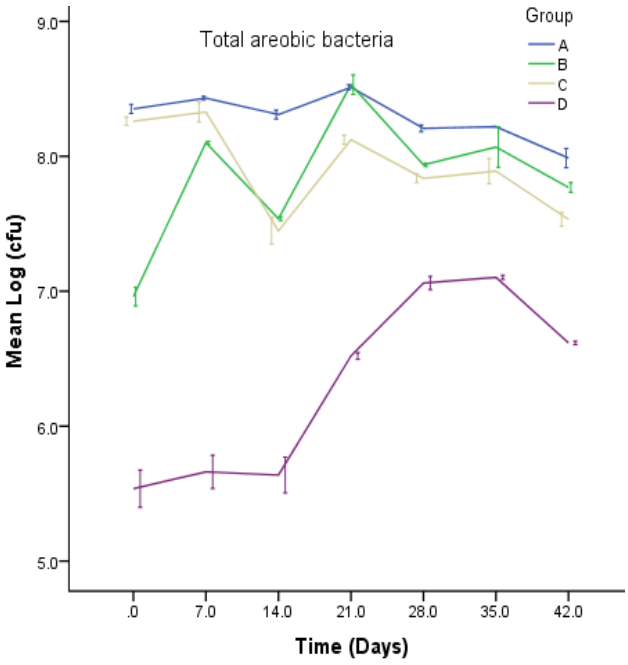

Figure 2. Total Count Bacteria of fresh oregano samples during 42 days at room temperature (A=8.28^{a}, B=7.91^{ab}, C=7.84^{b}, D=6.30^{c}, P<0.05) Different letters indicate significant differences (p<0.05). The separation of means (Tukeys test) was carried out based on the pooled effect of treatments during the whole storage period.

The anaerobic bacteria growth for four groups during the storage period was illustrated in (Figure 3). Different initial microbial loads for each group have been observed. Group D had significantly the lowest anaerobic bacteria growth which may be attributed to lactic acid as the main ingredient in this group. In general, the growth pattern of anaerobic bacteria during the storage period was similar to the growth pattern of aerobic bacteria.

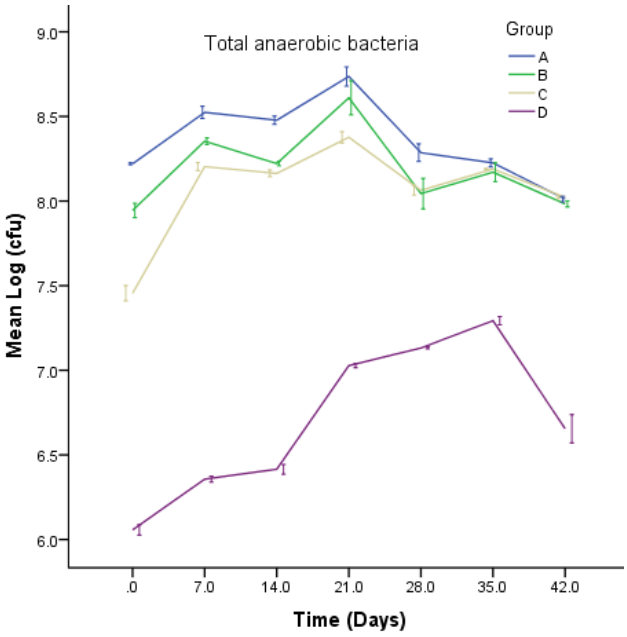

Figure 3. Anaerobic bacteria growth of green thyme samples during 42 days at room temperature (A=8.35^{a}, B=8.18^{ab}, C=8.06^{b}, D=6.74^{c}, P<0.05). Different letters indicate significant differences (p<0.05). The separation of means (Tukeys test) was carried out based on the pooled effect of treatments during the whole storage period.

The growth of psychrotrophic bacteria during storage period was shown in (Figure 4). It was found that there were significant differences in the growth of psychrotrophic bacteria between groups during the storage period (35 days). Similar to the results of total aerobic and anaerobic bacteria; Group D exhibited significantly the lowest psychrotrophic bacteria count (3.35 log). It was found that the addition of lactic acid in group D led to very low ultimate pH (3.5-4.5) during whole period of storage. This sharp decline in pH had an effect on microbial growth was probably caused by physiological and morphological changes in bacterial cells. Other studies found that lactic acid could completely prevent the growth of *Salmonella enteritidis, Escherichia coli and Listeria monocytogenes* by disruptive action on the content and activity of bacterial proteins [16]. Moreover, cytoplasmic membrane, membrane structure and intracellular structures were damaged by lactic acid [17,18]. As a general conclusion, it was clear that the most effective ingredients against aerobic, anaerobic, and psychrotrophic bacteria were lactic acid. Moreover, the growth of aerobic and anaerobic population was very similar, and this finding showed that most probably it was the same microbial population that can grow both in the presence or absence of oxygen, like for example lactic acid bacteria. On the other hand, the growth curves of aerobic and anaerobic microorganisms in group D (pH 4.4) showed that part of the population was aciduric and acidophilic, because it resisted to the low pH and after 14 days there was a sensitive increase (about 1.5 log cycles) of the bacterial load. The results of psychrotrophic population showed that these microorganisms can grow during the first 7 days but the final load was significantly different from the starting point.

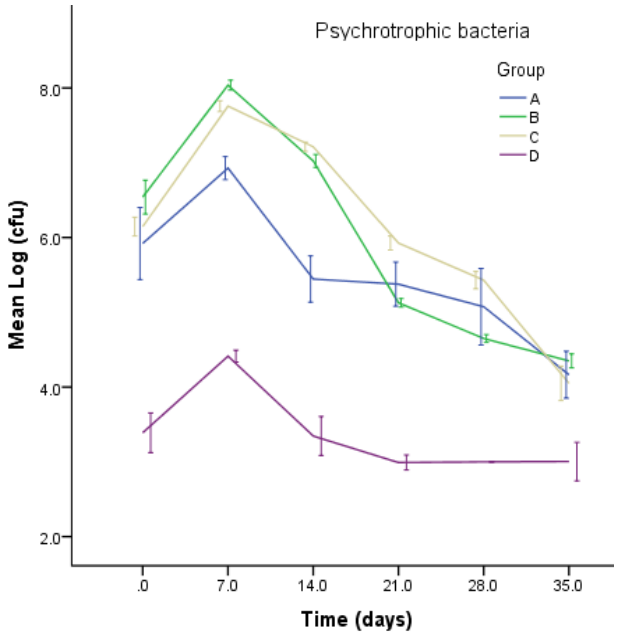

Figure 4. Psychrotrophic bacteria growth of oregano samples during 35 days at 4°C (A=5.48[a], B=5.95[a], C=6.08[a], D=3.35[b], P<0.05). Different letters indicate significant differences (p<0.05). The separation of means (Tukeys test) was carried out based on the pooled effect of treatments during the whole storage period.

The growth behavior of yeasts and molds during the storage period (42 days) of fresh oregano was shown in (Figure 5). There were no significant differences in the initial count of yeasts and molds for all four groups. Generally, all groups exhibited similar growth pattern during the whole period of storage. This result may be attributed due to the ability of various yeasts and molds to grow in a wide range of pH (2-9) and a broad temperature range (5 to 35°C) [19].

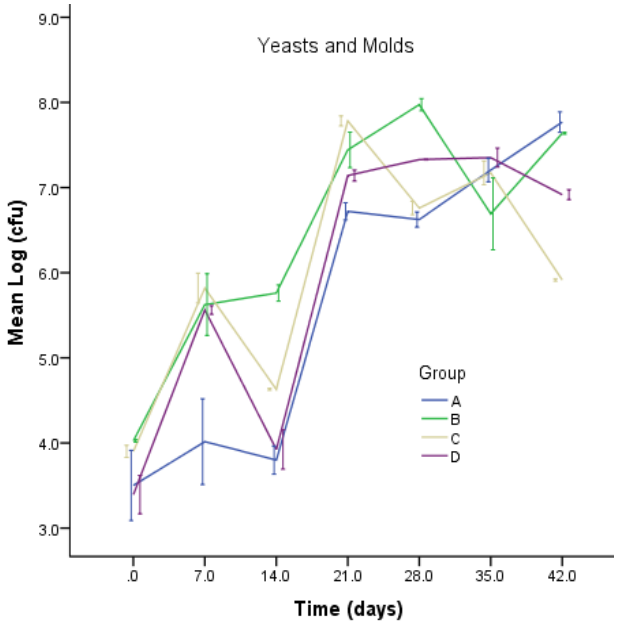

Figure 5. Mold and Yeast growth in fresh oregano during 42 days (A= 5.66, B=6.45, C=6.00, D=5.94, P=0.222). The separation of means (Tukeys test) was carried out based on the pooled effect of treatments during the whole storage period.

3.3. Color Attributes

During the storage period (42 days), color attributes (*L**, *a**, *b**) have been measured to investigate the effect of treatments on color attributes. *a**-values for all groups during the storage period are shown in (Figure 6). In general, there were slight significant increases in *a**-values during storage for all groups except in some cases. Group C exhibited significantly higher a-values than other groups. The lowest change in redness values during the storage period if compared to others. This can be attributed due to the addition of sumac which contained high contents of natural pigments such as anthocyanins that contribute to red-blue color [20]. On another hand, the increase in *a**-value in all groups during storage may be attributed to chlorophyll degradation which is a symptom of the transition of chloroplasts to gerontoplasts, score characteristics of plastids of aging. Degradation of chlorophyll appeared to the surface pre-existed colors, in this case, carotenoids [21].

One of the main causes of colour changes in oregano leaves may be attributed due to enzymatic browning reactions that produce oxidative reactions of phenolic compounds by polyphenol oxidase [22]. The degradation of pigments depends on many factors including storage environment, pH, duration of the storage period, and the concentration of natural additives that applied after harvesting [23] as well as chlorophyll content of leaves [24].

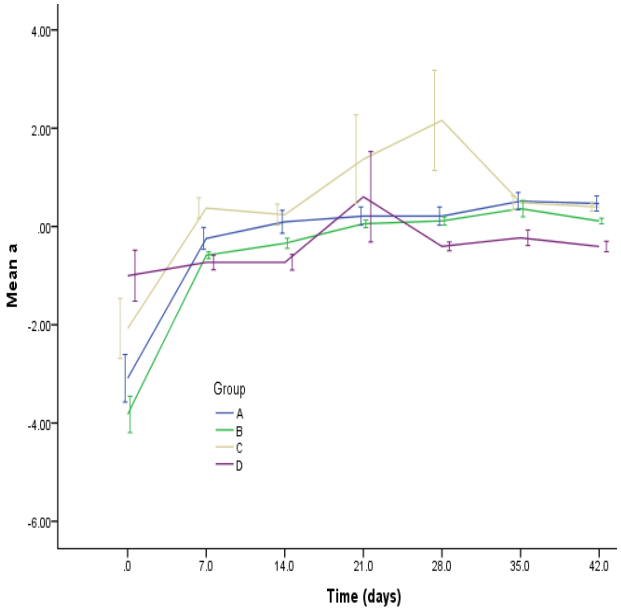

Figure 6. a* values of fresh oregano during the storage period (42 days) (A=-0.26[ab], B=-0.59[b], C=0.42[a], D=-0.41[b], P<0.05). Letters indicate significant differences (p<0.05). The separation of means (Tukeys test) was carried out based on the pooled effect of treatments during the whole storage period.

*b**- values for all groups during the storage period are shown in (Figure 7). In general, there were slight significant increases in *b**-values during storage for all groups in particular in the first week of storage. In particular, Group B and D exhibited significantly higher b*-values if compared to group A and C. b*- values are usually affected by chlorophyll degradation which means that group B and D exhibited quite higher chlorophyll

degradation than group A and C. In general, different factors that affect chlorophyll degradation. In this context, a study examined the effect of citric acid on the colour of fresh-cut rocket and swiss during 12 days of storage in both dark and light conditions. It was found that the treated samples with citric acid did not show any effect in preventing chlorophyll degradation during light storage [22].

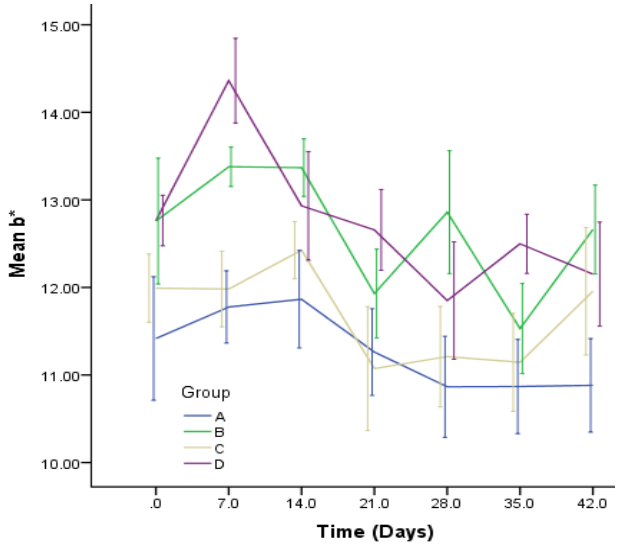

Figure 7. b* values of fresh oregano during the storage period (42 days) (A=11.27[b], B=12.64[a], C=11.68[b], D=12.74[a], P<0.05). Letters indicate significant differences (p<0.05). The separation of means (Tukeys test) was carried out based on the pooled effect of treatments during the whole storage period.

*L**- values for all groups during the storage period are shown in (Figure 8). In general, there was a slight significant decrease in *L**-values during storage for all groups. It was found that the highest *L**-values during the whole period of storage were observed in group A if compared to other groups. This can be attributed due to the addition of ingredients such as onion, sumac, and lactic acid that may be contributed to darken the color of oregano leaves.

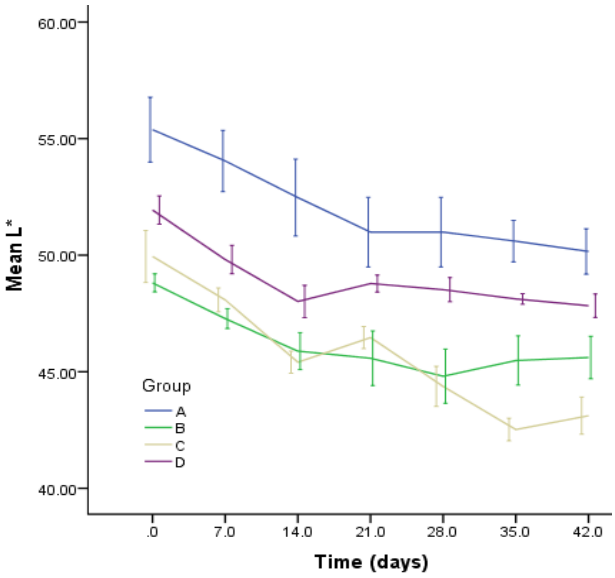

Figure 8. L* values of fresh oregano during the storage period (42 days) at room temperature (A=52.09[a], B=46.21[c], C=45.70[c], D=49.00[b], P<0.05). Letters indicate significant differences (p<0.05). The separation of means (Tukeys test) was carried out based on the pooled effect of treatments during the whole storage period.

In this study, microbiological challenge testing was applied to study the ability of the growth of *Clostridium sporogenes DSM 795*. It is used to mimic *Clostridium botulinum* growth, in both the recipes that used in B and D treatments using agar journey (spot, spreading methods) and sensitivity methods.

In both methods, the result demonstrated that the recipe of treatment D (containing onion, oil, salt, and lactic acid) could be resisted the growth of *Clostridium sporogenes* DSM 795. This result may be due to the presence of lactic acid that reduced pH to 4.4. It has been traditionally accepted that spores of *Clostridium botulinum* will not germinate and grow at pH 4.8 or below [26]. On another hand, the components of group B containing only onion, salt, and oil were unable to prevent bacterial growth as shown in photos (1, 2, 3).

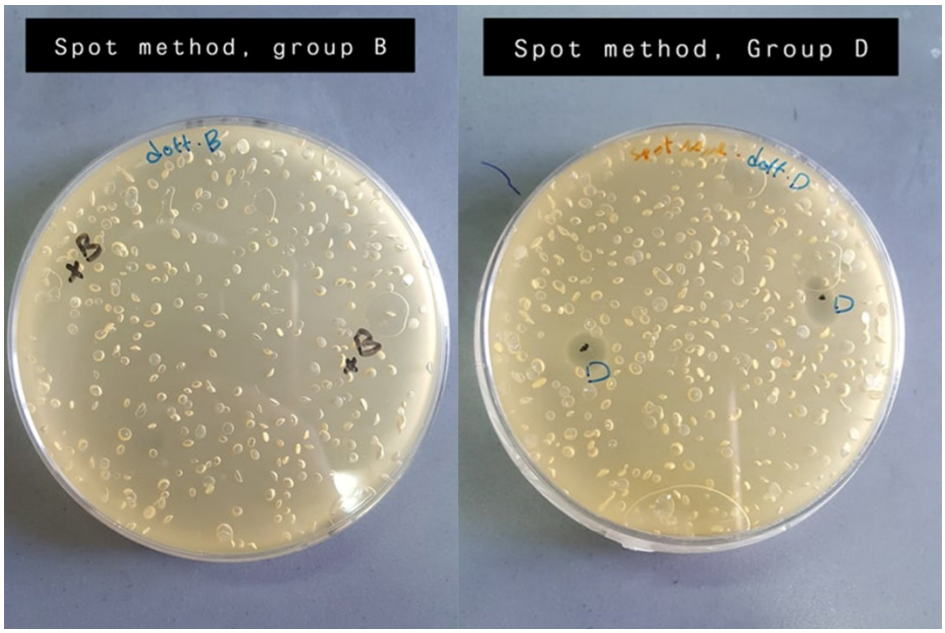

Photo 1. Growth of *C. Sporogenes* in group B and D recipes by using the spot method

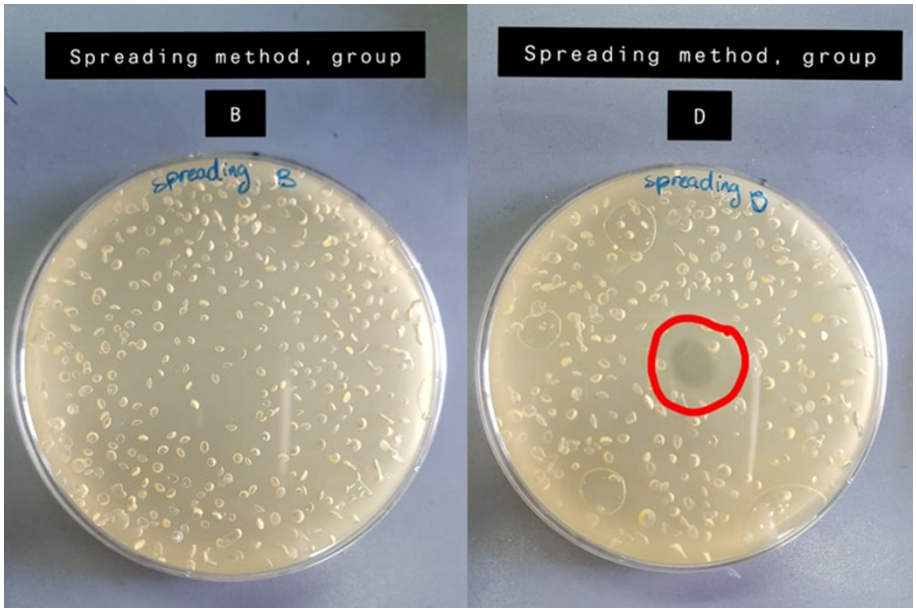

Photo 2. Growth of *C. Sporogenes* in group B and D recipes by using the spreading method

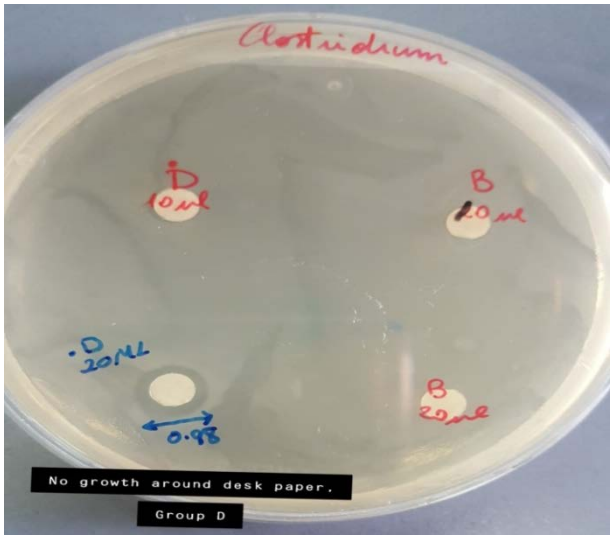

Photo 3. Growth of *C. Sporogenes* in group B and D recipes by using the sensitive test

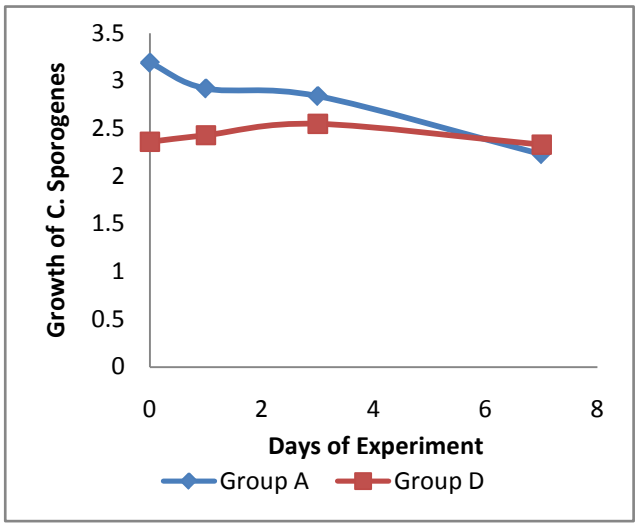

Figure 9. Microbiological Challenge test in group A and D green thyme leaves for 7 days

The growth of *Clostridium sporogenes* on fresh oregano leaves for 7 days was shown in Figure 9. Group A (Control) and D (with lactic acid) were selected to study the potential growth of *C. Sporogenes* in fresh oregano leaves which were stored at room temperature 25°C for 7 days. The results showed that group A could decrease growth of *C. Sporogenes* during the storage period if compared to group D. Oregano is rich in essential oils and antimicrobial agents such as thymol, carvacrol, geraneol, and borneol which may affect the growth of bacteria such as *C. sporogenes*. On another hand, lactic acid is a common preservative substance that used to increase the shelf life of products [27].

In conclusion, it was possible to employ hurdle technology to obtain fresh oregano products that were stable for reasonable shelf life. Different ingredients in fresh oregano leave preparations were employed during this study, but it was found that lactic acid was the most effective to keep different quality traits during storage.

References

[1] Skoula, M., & Harborne, J. B. (2002). The taxonomy and chemistry of Origanum. Oregano. In: The Genera Origanum and Lippia, (Kintzios SE, ed.). CRC Press, Boca Raton, FL, 67-108.

[2] Arcila-Lozano, C. C., Loarca-Piña, G., Lecona-Uribe, S., & González de Mejía, E. (2004). El orégano: propiedades, composición y actividad biológica de sus componentes. Archivos Latinoamericanos de nutrición, 54(1): 100-111.

[3] Ibrahim, S. K., Ibrahim, L., Ismail, A., Basal, A., Kayal, M., Ghanem, H., & Rammel, S. (2011). Differentiation of different species of Origanum and Thymus using proteins and isoenzymes profile. International Journal of Botany, 7(4): 283-288.

[4] Salehi, B., Mishra, A. P., Shukla, I., Sharifi-Rad, M., Contreras, M. D. M., Segura-Carretero, A.,. & Sharifi-Rad, J. (2018). Thymol, thyme, and other plant sources: Health and potential uses. Phytotherapy Research, 32(9): 1688-1706.

[5] Xu, H. (2006). Delling M, Jun JC, and Clapham DE. Oregano, thyme and clove-derived flavors and skin sensitizers activate specific TRP channels. Nat Neurosci, 9: 628-635.

[6] The Turkish Origanum species S.E. Kintzios (Ed.), Origanum, the genera and Origanum and Lippia, London and New York, Taylor and Francis (2002), pp. 109-126.

[7] Hazzit, M., Baaliouamer, A., Faleiro, M. L., & Miguel, M. G. (2006). Composition of the essential oils of Thymus and Origanum species from Algeria and their antioxidant and antimicrobial activities. Journal of Agricultural and Food Chemistry, 54(17): 6314-6321.

[8] De Palma, C., Falcone, S., Panzeri, C., Radice, S., Bassi, M. T., & Clementi, E. (2008). Endothelial nitric oxide synthase overexpression by neuronal cells in neurodegeneration: a link between inflammation and neuroprotection. Journal of Neurochemistry, 106(1): 193-204.

[9] Ocana-Fuentes, A., Arranz-Gutierrez, E., Senorans, F. J., & Reglero, G. (2010). Supercritical fluid extraction of oregano (Origanum vulgare) essentials oils: anti-inflammatory properties based on cytokine response on THP-1 macrophages. Food and Chemical Toxicology, 48(6): 1568-1575.

[10] de Souza, E. L., de Barros, J. C., de Oliveira, C. E. V., & da Conceição, M. L. (2010). Influence of Origanum vulgare L. essential oil on enterotoxin production, membrane permeability and surface characteristics of Staphylococcus aureus. International Journal of Food Microbiology, 137(2-3): 308-311.

[11] Salgueiro, L. R., Pinto, E., Goncalves, M. J., Pina-Vaz, C., Cavaleiro, C., Rodrigues, A. G., ... & Martinez-de-Oliveira, J. (2004). Chemical composition and antifungal activity of the essential oil of Thymbra capitata. Planta Medica, 70(06): 572-575.

[12] Ali-Shtayeh, M. S., Jamous, R. M., Abu-Zaitoun, S. Y., Akkawi, R. J., Kalbouneh, S. R., Dudai, N., & Bernstein, N. (2018). Secondary treated effluent irrigation did not impact chemical composition, and enzyme inhibition activities of essential oils from Origanum syriacum var. syriacum. Industrial Crops and Products, 111: 775-786.

[13] Barrett, D.M. and Lloyd, B. (2012), Advanced preservation methods and nutrient retention in fruits and vegetables. Journal of the Science of Food and Agriculture, 92 (1): 7-22.

[14] Montville, T.J., Matthews K.R. (2001). Chapter 2: Principles which influence microbial growth, survival, and death in foods. In: Doyle MP, Beuchat LR, Montville TJ, editors. Food microbiology: fundamentals and frontiers. Washington (DC): ASM Pr. p 13-32.

[15] Saxena, G., McCutcheon, A. R., Farmer, S., Towers, G. H. N., & Hancock, R. E. W. (1994). Antimicrobial constituents of Rhus glabra. Journal of Ethnopharmacology, 42(2): 95-99.

[16] Wang, C., Chang, T., Yang, H., & Cui, M. (2015). Antibacterial mechanism of lactic acid on physiological and morphological properties of Salmonella enteritidis, Escherichia coli and Listeria monocytogenes. Food Control, 47: 231-236.

[17] Vishaalini, C., Hema Shenpagan, N., & Lali G. (2016). Antibacterial activity on Medicinal herbs. Int. J. Urr. Microbiol. App. Sci., 5 (10): 814-819.

[18] Bhat, Z. F., Pathak, V., Bukhari, S. A. A., Ahmad, S. R., & Bhat, H. (2011). Quality changes in Chevon Harrisa (meat based product) during Refrigerated Storage. Int. J. Meat Sci., 1(1): 52-61.

[19] Rostro-Alanis, M. D. J., Báez-González, J., Torres-Alvarez, C., Parra-Saldívar, R., Rodriguez-Rodriguez, J., & Castillo, S. (2019). Chemical composition and biological activities of oregano essential oil and its fractions obtained by vacuum distillation. Molecules, 24(10): 1904.

[20] Shabbir, A. (2012). Rhus coriaria linn, a plant of medicinal, nutritional and industrial importance: A review. J Anim Plant Sci., 22(2): 505-12.

[21] Brar, J.K., Rai, D.R., Singh, A. et al.(2013) Biochemical and physiological changes in Fenugreek (Trigonella foenum- graecum L.) leaves during storage under modified atmosphere packaging. J Food Sci Technol, 50 (4): 696-704.

[22] Ferrante, A., Incrocci, L., Maggini, R., Serra, G., & Tognoni, F. (2004). Colour changes of fresh-cut leafy vegetables during storage. J. Food Agric. Environ., 2(3&4): 40-44.

[23] Manolopoulou, E., & Varzakas, T. (2016). Effect of temperature in color changes of green vegetables. Current Research in Nutrition and Food Science Journal, 4 (Special Issue Nutrition in Conference October 2016): 10-17.

[24] Kaur, P., Rai, D. R., & Paul, S. (2011). Quality changes in fresh-cut spinach (Spinacia oleracea) under modified atmospheres with perforations. Journal of food Quality, 34(1): 10-18.

[25] Tan, C. T., & Francis, F. J. (1962). Effect of processing temperature on pigments and color of spinach. Journal of Food Science, 27(3): 232-241.

[26] Lalitha, K. V., & Gopakumar, K. (2005). Influence of temperature and pH on growth and toxin production from spores of Clostridium botulinum. Journal of Aquatic Food Product Technology, 14(2): 39-50.

[27] Tayyba, G., Muhammad, I., Zahid, A., Tahir, A., Zubia, Z., Asma, T., Muhammad, K., Nudrat, E., & Sajid M. (2014) Recent trends in lactic acid biotechnology: A brief review on production to purification. J. Radiat. Res Appl. Sc., 7(2): 222-229.