



Research Article

Properties of Lemon Oil and its Effect on Some Characteristics of Soft Milk Drink (Al-Shenina)

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Abstract | The aimed of this study was note the effect of adding lemon oil at (0, 0.50, 1 microliter of lemon peel oil/liter of diluted yoghurt drink and to observe the effect of this on the sensory, microbial properties during storage at a 5°C for 21 days at Taking readings every seven days, the results showed that the properties of cold-extracted lemon oil were (6.50 mg KOH/L, 52.6 mEq/1 kg of oil, 0.920, 1.154 centipoise, 120 °C) for pH. Peroxide number, specific gravity, relative viscosity and smoking point, respectively. As for the results of the sensory properties of the product during storage During the storage period, a gradual increase in acidity values was observed throughout the first seven days. This rise may be attributed to ongoing microbial activity and biochemical changes occurring within the product, which contribute to the development of acidic compounds over time, and the highest values in the comparison sample reached 0.27% compared to 0.2 and 0.17% for the milk drink samples containing 0.5 and 1. µL/L respectively, followed by a non-significant State the P-value used to determine statistical significance As for the microbial properties of the product, it showed no There was growth in the values of the total number of coliform bacteria, yeasts, and molds. As for the estimated numbers of lactic acid bacteria, it was shown that there was a significant increase in the values during the first seven days of storageThe highest acidity values were recorded in the control treatment, reaching 51×10^{-4} , compared to 45×10^{-4} and 37×10^{-4} in the treated samples. These values subsequently decreased toward the end of the storage period. Furthermore, the results of the sensory evaluation indicated a higher preference for the sample containing 0.5 µL/L of lemon peel oil, which achieved a score of 81% at the end of the storage period, compared to the other samples.

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Keywords | Limon peels oil, Lactic acid bacteria, Acid number, Diluted yogurt drink



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Introduction

Waste recycling is one of the most important methods highlighted in research for the purpose of obtaining new environmentally friendly

products, and essential oils are one of these materials, which are active chemical compounds characterized by being materials extracted from citrus peels and described as active chemical compounds that inhibit the growth of many organisms. Microscopic ones

milk is considered the least adulterated form among dairy products and is produced by fermenting pasteurized milk using lactic acid bacteria. In addition to being natural antioxidants, these bacteria exhibit beneficial effects, particularly against pathogenic microorganisms. As for Shenina, it is one of the dairy drinks consumed in Iraq, Turkey and Iran (Koksoy and Kilic, 2003). Essential oils can affect the activity of starter bacteria during storage, and that consumption of large quantities of these products indicates achieving a better lifestyle because this product contains lactic acid bacteria, which are described as vital enhancers. (Mohmoudi *et al.*, 2012), and Shehata *et al.* (2020) indicated in their study that the use of essential oils in foods is an appropriate method to reduce the effect of preservatives on food quality characteristics.

Limonene, which is the active ingredient in lemon oil, constitutes about 32-98% of the active substances that make up lemon oil, which are monocyclic terpenes (Gobato *et al.*, 2015).

Materials and Methods

Oil extraction: The oil was extracted using the hydrodistillation method using a clavenger unit, and based on what was stated in (Rasouli *et al.*, 2021), the oil samples were kept in opaque glass containers under refrigeration until analysis of their physical and chemical properties was conducted and the research was carried out.

Chemical and physical properties of the oil

- **Relative density:** It was estimated using a 20 ml density bottle and according to the method (Agapin, 2017)
- **Acidity value:** The total acidity of the oil was measured according to AOAC (2000).
- **Peroxide value:** The peroxide number of the oil was estimated based on what was stated in AOAC (2008).
- **Relative viscosity** was determined following the method described (IUPPC, 1991), using a 20 mL pipette to measure the time required for the oil to drain, followed by a similar measurement using water for comparison.
- **Smoking point:** It was estimated according to what was stated in Firestone (2009) by placing an amount of oil on a hot metal surface and calculating the temperature at which white smoke begins to appear using a thermometer.

Manufacture of diluted yoghurt drink (Shenina) flavoured with lemon oil. The diluted yogurt drink was manufactured according to the method mentioned (Younis, 2022). Different concentrations of lemon peel oil were added (0, 0.5, 1) microliters/liter of the diluted yoghurt drink were added, then all samples were stored in the refrigerator temperature was 5°C for 21 days, and tests were taken every 7 days for 21 days.

Chemical tests during storage

- **Estimation of the peroxide number:** The values of the peroxide number in the product were estimated according to the AOCS Method (2009) Cd-8-53
- **Estimation of total acidity:** The percentage of total acidity in the product was estimated according to the method mentioned before (Hool *et al.*, 2004).

Microbial tests

- **Estimating the total number of lactic acid bacteria:** After preparing the cuttings and preparing the nutrient medium (Man Rogosa Sharpe media) (MRS), the plates were poured, inoculated with samples, and incubated at 37°C for 48 hours under anaerobic conditions. Lactic acid bacteria were calculated by multiplying (number of colonies × reciprocal of dilution) (Abdelsalam and Akdo, 2023)
- **Estimating the number of coliform bacteria:** The presence of coliform bacteria was determined using MacConkey Agar medium, following the method described by Younis (2022), 3. Estimating the numbers of yeasts and molds using Potato Dextros Agar, growing colonies were counted at 37 °C for 48 hours (Difco, 1984).

Sensory evaluation

The diluted yoghurt drink with added concentrations of lemon oil was sensory evaluated during the 21-day storage period according to the model proposed (Younis, 2022).

Statistical analysis

The results were analyzed statistically according to the CRD randomized design system and according to the (SAS, 2001) program.

Results and discussion

Table 1 shows some of the physical and chemical properties of lemon peel oil. From it, we note that

the specific gravity, relative viscosity, and smoking point reached 0.920 and 1.154 centipoise at 120 °C, respectively. The acidity number reached 6.50 mg koH per gram of oil, and the peroxide number reached 52.6 m Eq. Oxygen per kg of oil, and the results obtained are consistent with what was indicated by Etta-Francis *et al.* (2022) and Al-Obaidi (2023) in their studies on the same topic.

Table 1: *Some physical and chemical properties of lemon peel oil under experiment.*

The attribute	Value
Relative density	0.920
Relative viscosity (centipoise)	1.154
Smoking point (°C)	120
Acid Value (mg KoH/g)	6.50
Peroxid Value (O2 meq/kg)	52.6

Table 2 shows the total acidity values of the milk drink upon storage, and from it we notice a non-significant increase in the values until the first week of storage. It is worth noting that the total acidity of the comparison sample was higher than the acidity of the other treatments, which shows the effect of the essential oil on bacterial growth, in particular Lactic acid bacteria (Ambrosio *et al.*, 2019), The highest values were observed in the control treatment after 7 days of storage, amounting to 0.27%, compared to 0.20% and 0.17% for the treatments containing 0.50 and 1 µmol/L, respectively. The lowest values were recorded on the first day of storage. This indicates a

gradual increase in the measured parameter over the storage period, with the control treatment showing the highest levels, while the treatments with added concentrations exhibited relatively lower values.

It reached 0.12% for all Transactions. These obtained results are consistent with what was stated by Thabet *et al.* (2014) that the number of lactic acid bacteria has a role in the development of acidity. It must also be noted that the diluted milk product fell within the limits of the standard specification (CXS3332R) for the year 2018 regarding diluted milk drink, which states: That the total acidity exceeds 0.7%.

Table 3, we notice that the peroxide number values for the milk drink during storage, where the values increased non-significantly during the storage period, and the highest values were for the comparison treatment at the end of the storage period, which amounted to 5.56% mg/kg oil, and the lowest for the treatments in 1 day of storage, which amounted to 4.48. For all transactions.

From the above, it becomes clear to us the effect of lemon oil as an antioxidant compared to the control treatment, where We can say that it worked to reduce oxidation reactions, which reduced the value of the peroxide number when compared to the control treatment, especially at concentrations of 1 microliter/liter. These results are consistent with what was indicated by (Farahmandfar *et al.*, 2018), who stated that the essential oil, when added at concentrations of

Table 2: *Total acidity values for diluted yogurt drink to which concentrations of lemon peel oil were added during storage.*

The amount of oil added is in microliters/ litre	1day	7 day	14 day	21 day	Effect of addition ratio
Standard sample	0.12±0.05b	0.27±0a	0.26±0.05ab	0.26±0.02ab	0.227a
0.5	0.12±0.05b	0.20±0.07ab	0.18±0.11ab	0.17±0.02ab	0.167ab
1	0.12±0.05b	0.17±0.028ab	0.17±0.028ab	0.15±0.07ab	0.152b
Storage effect	0.12b	0.21a	0.20a	0.19a	

Table 3: *Peroxide values for samples of diluted yoghurt drink to which concentrations of lemon peel oil were added during storage.*

The amount of oil added is in microliters/ litre	1 day	7 day	14 day	21 day	Effect of addition ratio
Standard sample	4.88±0.11 c	4.95±0.07 c	5.3±0.14 B	5.56±0.04 a	5.176a
0.5	4.48±0.02 e	4.56±0.07 de	4.63±0.14 De	4.9±0.14 c	4.64b
1	4.48±0.14 e	4.5±0.14 e	4.56±0 De	4.75±0.07 dc	4.57b
Storage effect	4.61c	4.66c	4.83b	5.071a	

Table 4: Total number of lactic acid bacteria for diluted milk drink samples to which concentrations of lemon peel oil were added during storage.

The amount of oil added is in microliters/litre	1day	7 day	14 day	21 day	Effect of addition ratio
Standard sample	10±2.828 e	51±7.071 a	50±2.828 A	44±5.656 ab	38.75 a
0.5	10±2.828 e	45±7.071 ab	38±2.828 Bc	30±7.071 cd	30.75 b
1	10±2.828 e	37±4.424 bc	25±7.071 D	22±2.828 d	23.5 c
Storage effect	10c	44.33a	37.66b	32b	

1600 ppm, It can act as a better antioxidant than BHT in stimulating fat oxidation, so it can be an alternative to it. Likewise, Through the amount of added essential oil, we notice a clear difference between the control sample and the samples to which essential oil was added, as the peroxide number value decreased with increasing concentration of added lemon oil, reaching 4.64 and 4.57 compared to 5.76 for the control sample and 0.5 and 1 microliter per liter, respectively. Ibrahim (2021) and Zhong *et al.* (2018) have shown. Oxidation reactions are faster in the environmental environment due to the transfer of copper ions to the granule shell during coolant storage

Preparation of colon bacteria

No growth of *coliform* bacteria was detected in any of the treatments throughout the storage period. This can be attributed to the stringent sanitary conditions maintained during the manufacturing process, as well as the effectiveness of the heat treatment (pasteurization), which was sufficient to eradicate this bacterial species The milk was pasteurized at 85°C for 15 minutes, then cooled to 45°C, and then the essential oil was added at the concentrations used in the research (Zeena *et al.*, 2023).

Preparation of yeasts and molds

No growth of yeasts or molds was observed for all treatments because pasteurization was sufficient to eliminate them, as well as because health conditions were followed during manufacturing.

Total number of lactic acid bacteria

From Table 4, which indicates the total number of lactic acid bacteria. The comparison is 52×10^{-4} for all treatments. Sources indicate that the numbers of lactic acid bacteria increase during the first ten days of storage (Younis, 2022; Colakogh and Gusoy, 2011).

While Table 5, which includes the results of the sensory evaluation of yogurt drink samples treated with concentrations of lemon peel oil during storage,

it becomes clear to us at the end of the storage period that the residents preferred the samples treated with 0.5 microlitres/litre and obtained 81% after 21 days of storage. Compared with the control treatment, which obtained 41%, and the 1 microliter/liter treatment, which obtained 52.5% of the above, it can be said that lemon peel oil helped enhance the flavor of the diluted milk drink (Shenina) and It is preferred by consumers, in addition to improving the product's properties, and this is consistent with what was mentioned by Abdulsalam and Akdo (2023) and Abdulsalam and Alobidy (2024).

Table 5: Total sensory evaluation scores for samples of diluted yoghurt drink to which concentrations of lemon peel oil were added during storage.

The amount of oil added is in microliters/litre	day 1	7 day	day 14	Day 21
Standard sample	96.5	92.5	74.5	41
0.5	97.5	92	95	81
1	94	88.5	73	52.5

Conclusion

The research results we obtained show that the effect of lemon oil added to diluted yogurt drink stored in the refrigerator for 3 weeks was clear in the chemical properties (total acidity and peroxide number). Microbial by controlling the non-increase in total acidity was through the significant decrease in lactic acid bacteria, especially at the concentration of 1 microliter of lemon oil/litre. As for the sensory evaluation results, they showed consumers' preference for the sample containing 0.50 microliter of lemon oil/litre of diluted yoghurt drink compared to other samples at the end of the storage period references.

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Novelty Statement

Utilizing lemon peel residues and their content of essential oils as a natural preservative and achieving the principle of health sustainability.

Author's Contribution

Azhar I. Shuker: Implementation of the practical part.

Shaimmaa R. Abdulsalaam: Developing and writing the research plan.

Sumyia Kh. Badawi: Supervising the work.

Generative AI and AI-assisted technology statement

The authors declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

References

- AOAC, 2008. Official methods of analysis 16th Ed. Association of official Analytieal chemists international Arlinton, Virginia, U. S. A.
- AOCS, 2009. In: D. Firestone (Ed.), Official methods and recommended practices of the American Oil Chemists' Society (5th ed.). Champaign Press USA.
- AOAC, 2000. Association of official analytical chemists. Official Method of Analysis, 15th. ed., Washington DC, USA.
- Abdulsalam, S.R. and H.J. Akdo. 2023. Evaluation of addition peppermint oil on the microbial and sensory properties of a soft milk drink (Shenina). IOP J. Sci., 1213(1): 012078. <https://doi.org/10.1088/1755-1315/1213/1/012078>
- Abdulsalam, S.R. and A. Alobidy. 2024. The effect of using some extraction techniques on the physical and chemical properties of essential oils of citrus peels. NTU J. Agric. Vet. Sci., 4(1): 2788-9904. <https://doi.org/10.56286/ntujavs.v4i1.770>
- Agapin, J.S., 2017. Physico-chemical characterization of essential oil from peel and leaf of dalanghita (*Citrus nobilis*). Int. J. Novel Res. Phys. Chem. Math., 4(2): 1-13. <https://doi.org/10.2139/ssrn.3624873>
- Al-Obaidi, A.A.A-M., 2023. Evaluation of the effectiveness of essential oils extracted from the peels of some local citrus species and their antibacterial effect. PhD thesis, Department of Food Sciences College of Agriculture and Forestry, University of Mosul.
- Ambrosio, C.M., N.Y. Ikeda, A.C. Miano, E. Saldaña, A.M. Moreno, E. Stashenko and E.M. Da-Gloria. 2019. Unraveling the selective antibacterial activity and chemical composition of citrus essential oils. Sci. Rep., 9(1): 17719. <https://doi.org/10.1038/s41598-019-54084-3>
- Colakoglu, H. and O. Gursoy. 2011. Effect of Lactic adjunct cultures on conjugated linoleic acid (CLA) concentration of yogurt drink . J. Food Agric. Environ., 9: 60-64.
- Difco, P.D.A., 1984. Detection and characterization of benomyl-resistant Monilinia laxa on apricots. Plant Dis., 29. <https://doi.org/10.1094/PD-69-29>
- Etta-francis, I.B.I.P.I.R.I.E.N.E., J. Akpa and E. Ehirim. 2022. Comparative study on the analysis and utilization of citrus peels essential oil and pectin.
- Farahmandfar, R., M. Asnaashari, M. Pourshayegan, S. Maghsoudi and H. Moniri. 2018. Evaluation of antioxidant properties of lemon verbena (*Lippia citriodora*) essential oil and its capacity in sunflower oil stabilization during storage time. Food Sci. Nutr., 6(4): 983-990. <https://doi.org/10.1002/fsn3.637>
- Firestone, D., 2009. Official methods and recommended partices of the AOCS.
- Gobato, R., A. Gobato and D.F.G. Fedrigo. 2015. Molecular electrostatic potential of the main monoterpenoids compounds found in oil Lemon Tahiti (*Citrus Latifolia* Var Tahiti). Parana J. Sci. Educ., 1(1): 1-10.
- Hool, R., D.M. Barbano, R. Bradley, M. Bulthaus, J. Lyncch and R. Reddy. 2004. Chemical and physical Methods. In: Standard methods for the examination of dairy products Wehr, H.M. AND Frank, J. F. (Ed). 17th ed Washington, American Public Health Association. Hum. Health J. Nutr., 15: 363-532.
- Ibrahim, R.A.A.A.R.I., 2021. Evaluation of the use of vegetable oils enhanced with the flavor of free fat in the manufacture of yoghurt. Master's thesis, Department of Food Science

- and Biotechnology, College of Agriculture and Forestry, University of Mosul.
- IUPPC, 1991. Standard methods for the analysis of oils, Fats and derivative. International Union of pure and Applide Chemistry. Commission on oils, Fats and derivative. A. Dieffenbacher and W. Pocklington ed. Blackweel Sci. Pub. Landon.
- Koksoy, A. and M. Kılıc. 2003. Effects of water and salt level on rheological propertiesof ayran, a Turkish yoghurt drink. Int. Dairy J., 13: 835–839. [https://doi.org/10.1016/S0958-6946\(03\)00103-1](https://doi.org/10.1016/S0958-6946(03)00103-1)
- Mahmoudi, R., H. Tajik, A. Ehsani, A.A. Farshid and P. Zare. 2012. Effects of *Menthalongifolia* L. essential oil on viability and cellular ultrastructure of lactobacillus casei duringripening of the probiotic feta cheese. Int. J. Dairy Technol., (In press). <https://doi.org/10.1111/j.1471-0307.2012.00867.x>
- Public Health Organization and Food and Agriculture Organization of the United Nations, 2018. Regional standard for dog syrup No. CXS, pp. 1-6.
- Rasouli, S.R., S.N. Ebrahimi and H. Rezadoost. 2021. Simultaneous ultrasound-assisted hydrodistillation of essential oil from aerial parts of the *Satureja khuzistanica* Jamzad and its antibacterial activity. J. Med. Plants, 20(80): 47-59. <https://doi.org/10.52547/jmp.20.80.47>
- SAS, 2001. Statistical analysis system uses guide. For personal computer, Release pp. 6-18.
- Shehata, S.A., E.A. Abdeldaym, M.R. Ali, R.M. Mohamed, R.I. Bob and K.F. Abdelgawad. 2020. Effect of some citrus essential oils on post-harvest shelf life and physicochemical quality of strawberries during cold storage. Agronomy, 10(10): 1466. <https://doi.org/10.3390/agronomy10101466>
- Thabet, H.M., Q.A. Nogaim, A.S. Qasha, O. Abdoalaziz and N. Alnsheme. 2014. Evaluation of the effects of some plant derived essential oils on shelf life extension of Labneh. Merit. Res. J. Food Sci. Technol., 2(1): 8-14.
- Younis, H.J.A., 2022. Study of the properties of peppermint oil and its effect on the chemical, microbial and sensory characteristics of diluted milk drink (Shenina). Master's thesis, Department of Food Sciences, College of Agriculture and Forestry, University of Mosul.
- Zeena, D.A., R.A. Shaimaa and S.A. Khazal. 2023. The effect of adding grape seed oil on the chemical, physical and microbial properties of yoghurt. In: IOP conference series: Earth and environmental science. IOP Publishing. 1158(11): 112007. <https://doi.org/10.1088/1755-1315/1158/11/112007>
- Zhong, J., R. Yang, X. Cao, X. liu and Qin. 2018. Improved physicochemical properties of yoghurt fortified with fish oil oryzanol by Nanoemulsion technology. Molecules, 23(56): 10-3390. <https://doi.org/10.3390/molecules23010056>