



Organic Cottage Cheese Reliability Maintenance Using Oregano Essential Oils

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Abstract

Cottage cheese is a very common coagulated dairy product, although, like other dairy items, it has a short shelf life. Thymol and four varieties of Argentinian oregano essential oils were used to enhance organic cottage cheese. It was examined whether chemical markers for oxidation of lipids, oxidation, and variations in the profiles of fatty acids and organic acids occurred throughout the course of 30 days of heat storage. The conjugated dienes in the Cordobes EO and thymol-flavoured samples were lower (15.95 and 15.54, respectively), whereas they were highest (21.10) in the control specimen (17.55). Owing to oxidation, the values of unsaturated fats in cottage cheese samples went down dramatically ($p < 0.05$). Samples that were flavoured with Cordobes and Compacto Eos made a lot less organic acid while they were being stored. When oregano essential oil is added to organic cottage cheese, it slows down the loss of quality during storage, which makes it last longer.

Keywords: Essential oil, Oregano oil, Shelf life, Cheese and Fatty acid

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1. Introduction

India makes up about 18.81% of the world's milk supply. In 2017-18 and 2018-19, the country will produce 176.35 million tonnes and 187.70 million metric tonnes of milk, respectively. It shows that India has a lot of potential and access to milk that can be used to make milk products. Indian people really like curdled dairy products like cottage cheese, chenna, sandesh, etc. But in India, cottage cheese is the most popular type of curdled milk. Cottage cheese is a dairy product made by coagulating sour milk, citric acid, or lactic acid with cow or buffalo milk, or a mix of the two. It shouldn't have more than 70% water, and milk fat shouldn't make up more than 50% of the total solids [1]. Cheese is one of the milk products that has the potential to operate as a good food matrix for the purpose of being a probiotic carrier. Cheese is a diverse type of food that is crucial to basic dietary nutrition owing to its natural high nutritional content, taste, and style. Cheese, when used as a probiotic delivery system, has a range of benefits over fresh fermentation process products such as yoghurt and fermented milk. These advantages include the fact that cheese has a higher pH, a more coordinating authority, and a significantly higher fat content, all of which provide greater protection for the probiotic bacteria that are contained within the cheese during storage and transit through the

gastrointestinal tract [2]. Similarly to other water solvents, dairy products are susceptible to hydrolyzing and oxidative rancidity. Microbial lipases catalyse the release of volatile fatty acids and their subsequent transformation to other acids and/or ethyl esters. After lipids are oxidised, hydroperoxides are produced that readily react with fat acids to produce secondary oxidation products, mostly aldehydes. During the cheesemaking process, organic acids are produced as byproducts of carbohydrate biotransformation and fat hydroxylation in milk, bacterial growth, or the addition of acidulants; these acids play an important role as flavour compounds, intermediate products, and metabolites in a wide range of biological mechanisms. They need to be quantified for dietary purposes and because their levels are a sign of bacterial activity [3].

Improved techniques for cheese protection have been created as a result of developing concern and awareness about the safety and quality of cheese. Investigations are being conducted into alternative preservation methods using natural substances. Spices and herbs are used frequently and are regarded as universally recognised as safe since they provide food a unique flavour and scent. Plant EOs in particular are gaining attention as potential preservatives and are well-liked by consumers.

EOs are complex, naturally occurring, volatile chemicals with a powerful odour. Certain organic compounds might possibly boost the overall quality of cheese by inhibiting oxidative rancidity, lowering or eliminating pathogenic bacterial survival, and extending the span time of cheese. The adoption of EO has only been the subject of a few investigations [4].

White soft cheese was preserved using CEO nanoemulsions at 0.50, 0.75, and 1.00 percent concentrations. Cheese preserved in 1.00% CEO nanoemulsion remained free of yeasts and moulds and psychrotrophic counts for up to 60 days of storage. Cheese maintained in 0.50 and 0.75% CEO nanoemulsion treatments showed low numbers of fungal species, as well as spoilage organisms bacteria [5]. By testing the overall acceptability qualities of cheese, it was discovered that cheeses conserved with 0.50 and 0.75% of CEO nanoemulsion solutions had the greatest overall ratings. The aim of that research was to find out what happened to the amount of *Staphylococcus aureus* in cow's milk when nisin and oregano essential oil (OEO) were added alone or together [6]. Next, the visual, physico-chemical, and microbiological qualities of FC made with milk containing nisin+OEO or nisin alone were compared to those of a control cheese. The findings demonstrated that adding nisin decreased the amount of *S. aureus* in milk in a manner similar to combining both nisin and OEO to the control, which resulted in a 0.001% reduction in pathogens and spoilage bacteria, fungus, and mould. [7]. In that investigation, the microbiological safety and sensory acceptability of cheese made with isolated EOs of ginger, clove, and thyme were evaluated. Ginger oil mostly consists of camphene and glycoside, according to preliminary phytochemical, whereas thymol and eugenol are the most prominent volatiles in thyme and clove, respectively [8]. The minimal inhibitory concentration (MIC) for EOs against 7 varieties of rust, yeast, and microbial pathogens made up 0.001% of the sample. Moreover, the EOs showed an intriguing safety profile, with CC50 values determine from 6.31 to 452.95 ng/mL for human hepatocellular carcinoma (HepG2) and from 729.4 to 841.66 ng/mL for regular person lung fibroblasts (WI-38) [9]. Results from a sensory analysis showed that fresh soft cheese made in the lab with added EOs was much more palatable than control samples ($P < 0.001$). Cheese from Minas Frescal was tested for its terpene content (particularly OVEO and ROEO), its ability to withstand in vitro digestion, and its physical and sensory properties [10]. Cheese's terpene content dropped across the board as its storage period lengthened. Although the addition of OVEO and ROEO slowed the growth of *L. acidophilus* LA-5 in cottage cheese, it had no effect on the bacteria's capacity to survive in the food under simulated gastric circumstances [11]. The presence of terpenes in cheese was significantly linked ($r = 0.82$) with the reduction in *E. coli* levels seen during the first 15 days of preservation. After longer periods of storage, OVEO and ROEO cheese received higher ratings for scent, taste, overall impression, and desire to buy [12]. The experiments have demonstrated that EOs from oregano and thyme are efficient against foodborne pathogens, including *E. coli*, *Lactobacillus*, *Salmonella*, and *S. aureus* that have been isolated from fermented meat items and cheeses. Yet, thyme and oregano

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essential oils (EOs) seem to limit the development of fungus, including *B. cinerea* and *Aspergillus* spp., during post-harvest, when these pathogens reduce the fruit's shelf life [13]. There is little evidence that sage or rosemary essential oils have any antibacterial effects. Meat, cream cheese, berries, and grapes were used in experiments to test the effects of adding various EOs and AMPs on extending the span of time for the ingredients. The activation strength and ductility of fat and proteins during storage were modest in cheese samples coated with sodium alginate integrated by the oils [14]. Moreover, low microbiological growth was found in cheese samples covered with multilayer, and the development of *Streptococci*, *Salmonella*, and *Investment* casting was entirely suppressed for all varieties of cheese. Also, the biofilm coating decreased the weight and toughness of the cheese when compared to the bare sample. In contrast to others, sensory study found that unprotected cheese, covered with alginate made of oil of *O. basilicum* were the most liked by panellists [15].

As an organically produced natural substance with shown antioxidant activity and food-preserving characteristics, oregano EO has the potential to be employed as organic preservative in perishable natural food items. This study set out to determine how the addition of Argentinean OEOs affected the lipid and basic acid profiles, as well as other quality indices, of organic cottage cheese (CC) subjected to an speeded up decomposition test.

2. Materials and methods

2.1 Processing of Essential Oils by Gas Chromatography

Origanum x majoricum, *O. vulgare* ssp. *Vulgare*, *O. vulgare* ssp. *hirtum* (clones Cordobes and Criollo), and Leaves and Flowers were sent to the Facultad Ciencias Agropecuarias. May saw the harvest of herbs that had been planted at their home. To produce oregano essential oil, samples of the plant's herbs and blossoms were distilled for two hours in a C-style device. A capillary column on an Agilent 6891 gas specialized roles equipped with a mass spectrometer and flame ionisation detection systems was used to determine the chemical composition.

2.2 Storage study

The four different oregano kinds' Eo and thymol were combined with CC and cooked for 8 minutes while mixing with an emulsifier. The addition of the Eo to the control sample was done in the same manner as the other samples. In order to conduct the tests, the CC was diverted to a 50 mL container and kept at 4°C for 30 days. Every 10 days, the observing quality parameters were measured: changes in the OA characterization, fatty acids, pH, total titratable acidity, conjugated dienes, and hydroperoxide value (HV).

2.2.1 Lipid hydroperoxides

The processing of the cheese specimen was done in order to measure the presence of lipid hydroperoxides. 5 mL of remineralization water and 1 g of cream cheese were combined by Ultraturax and blended for 45 seconds. Fatty hydrides were precipitated into a 10 mL solution of 1:1 methanol to formaldehyde. The specimens were extractor for 10 minutes at gram 1000. Here, 1 mL of the iron

(II)/thiosulfate solution was added to 1 mL of the dichloromethane phase in a glass that had been washed with chloroform. During magnetic stirring, BaCl₂ was vigorously added to 50 mmol/L FeSO₄, along with 2 mL of 10 mol/L HCl. The result was then filtered to remove precipitated. In accordance with the IDF standard (74A:1991), 500 mL of this result and 500 mL of 3.94 mol/L NH₄SCN was included to 49 mL of methanol:clf. At 500 nm, absorbance was determined, and noise was subtracted at 700 nm.

The specimen of cheese (25 g) was put into a container. After that, 45 mL of CM (3:1 mL) were added this process is done for extraction of lipid after that the lipids were taken out and then mixed with 6 mL of hexane. At 233 nm, the absorption rates of the conjugated dienes were evaluated. The sample extinction coefficient E was given as the conclusion.

2.2.2 Analysis of Fatty acid methyl esters (FAME)

Transmethylation of isolated fat using a 30 g/L mixture of sulfuric acid in methanol produced FAME. In order to determine which fatty acid compounds were present in the total lipids, an Agilent Framework 6891 Series, Mass Selective Analyzer, Agilent 5973 Connectivity was used in conjunction with a flame ionisation predictor. Utilized was an EconoWax capillary column. The temperature of the column climbed to 200 C at a rate of 10 C/min, and then from 200 to 250 C at a range of 5 C/min. The carrier gas was nitrogen (rate of flow is 1 mL/min). The FAME were discovered by comparing their persistence durations to those of actual specimen acquired from Sigma Chemical Co. Internal standard for quantitative fatty acid measurement was HAME.

2.2.3 Analysis of PH and Total Titratable Acidity

Prior to measuring the pH, samples of the standardised CC with an Ultrataurux in water (1:8) for 59 seconds. Using a digital pH metre, the pH of a 10-gram homogenised cheese sample was measured. TTA was evaluated by titration with 0.1 NaOH while stirring continuously until the acid concentration was neutralised and a uniform pink colour developed. The calculation for the quantity of acid created during yeast was as follows:

$$\text{Lactic Acid (g} \div \text{kg)} = P * 0.1 * 0.008 \quad (1)$$

where P is the amount of NaOH needed to neutralise the acid, and the normalcy of the NaOH used is 0.1 (molequi/L).

2.3 Statistical Analysis

During the research data was collected in triplicate (n=3) and ANOVA and test for statistically significant variations between means uses the LSD Fisher's numerous range test. The equations for the LR for the variables observed on the time of the CC preservation research were derived.

3. Results and Discussions

3.1 Antioxidant activity by FRAP method

A decrease in the ferric di complex was seen in the FRAP assay when any of the four EOs tested here were applied. An excellent method for measuring antioxidants' electron-donating potential is the reducing power test. To a large extent, the ferric reducing activity of phenolic compounds is determined by the structure of their conjugated twin bond systems and their hydroxyl functionalities. This feature was seen in samples that included a greater concentration of CDB-containing monoterpenes such "o-cimene, cymene, a-terpinene, and thymol". Higher AEAC was found in Criollo and Cordobes. The peak concentration of thymol in each EOs, as well as the showing of o-cymene and terpinene, may explain these findings. Mendocino EO, on the other hand, had the lowest thymol content, making it the sample with the lowest value.

3.1.1 Lipid hydroperoxides

The HPV of natural cottage cheese samples that had oregano essential oil applied during the course of 30 days of preservation at 40 C shown in figure 1. Antioxidant addition and storage duration both had an impact on the buildup of hydroperoxides. In general, the hydroperoxide value dramatically rose throughout the course of storage. Over the three periods considered, under the mean of every specimen found for HPV was vary from the control. Only the differences between the control and the other treatments were found to be statistically significant at storage days 10 and 20 (p=0.0082). All flavours of organic CC had different HPV at storage day 30, and this comparison was statistically significant (p=0.0136). The control sample had the greatest hydroperoxide value (3.93 meqO₂/kg), whereas the sample containing thymol had the lowest value (2.46 meqO₂/kg), which was then followed by the specimen containing Criollo EO. Furthermore, it was shown that at storage day 30, there were no significant differences between the "CC-COR, CC-CRIO, CC-MEN, and CC-THY" specimens. In the following treatment sequence, the fat peroxidation response in natural cheese reduced. It is clear from this sequence that thymol served as the highest antioxidant substance. The natural antioxidant properties of mint, ginger, and beet extracts, as well as their mixtures, is greater than that of the artificial antioxidant BHA and BHT found in milk desert sandesh. The significance of utilising antioxidants in preventing oxidation process in milk items is shown by this outcome. Hydroperoxides were not found in cheese that included green tea extract. Even low-fat variants of dairy products include lipids, underscoring the need for study into the by-products of lipid oxidation and how to avoid them.

3.1.2 Conjugated dienes

All specimens of organic CC were analysed by spectrophotometry, and the results revealed that conjugated dienes accumulated significantly (p 0.001) from day one to day forty-one of storage at 40 C displayed in figure 2. Not until day 20 of storage did we find any significant differences between the control and experimental specimens of CC. Conjugated dienes were lowest in CC-COR and CC-THY after 30 days in storage, with CC-C

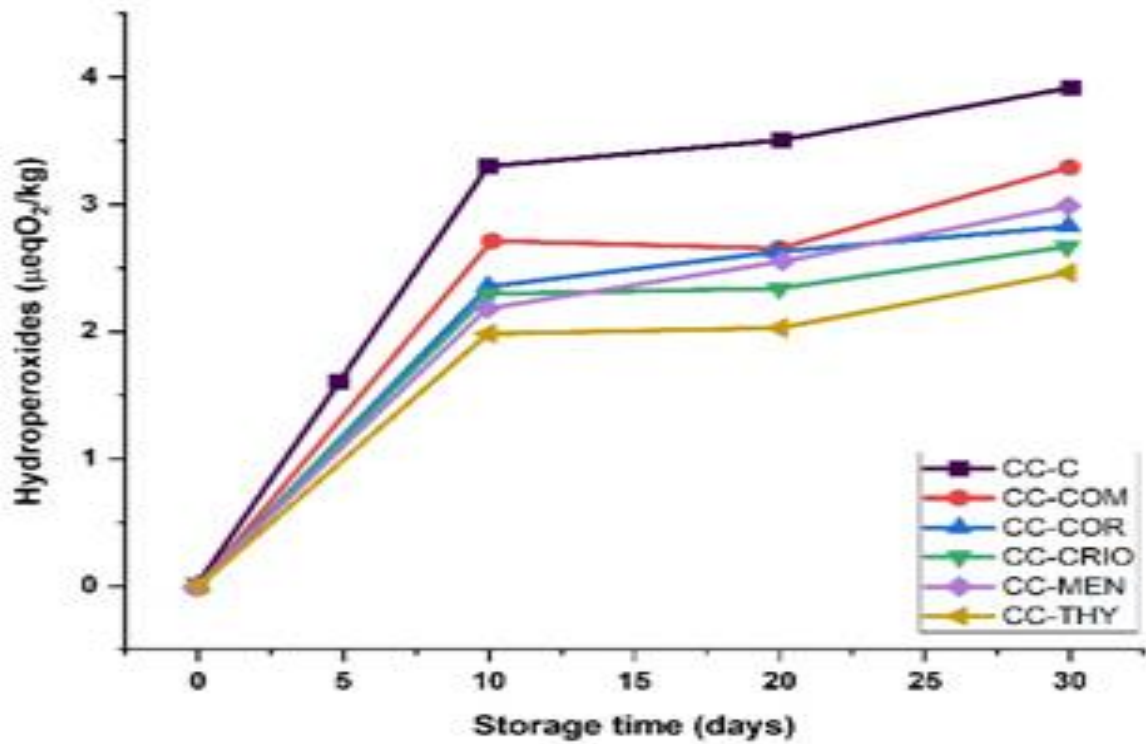


Figure 1: Graphical Diagram for Hydroperoxides

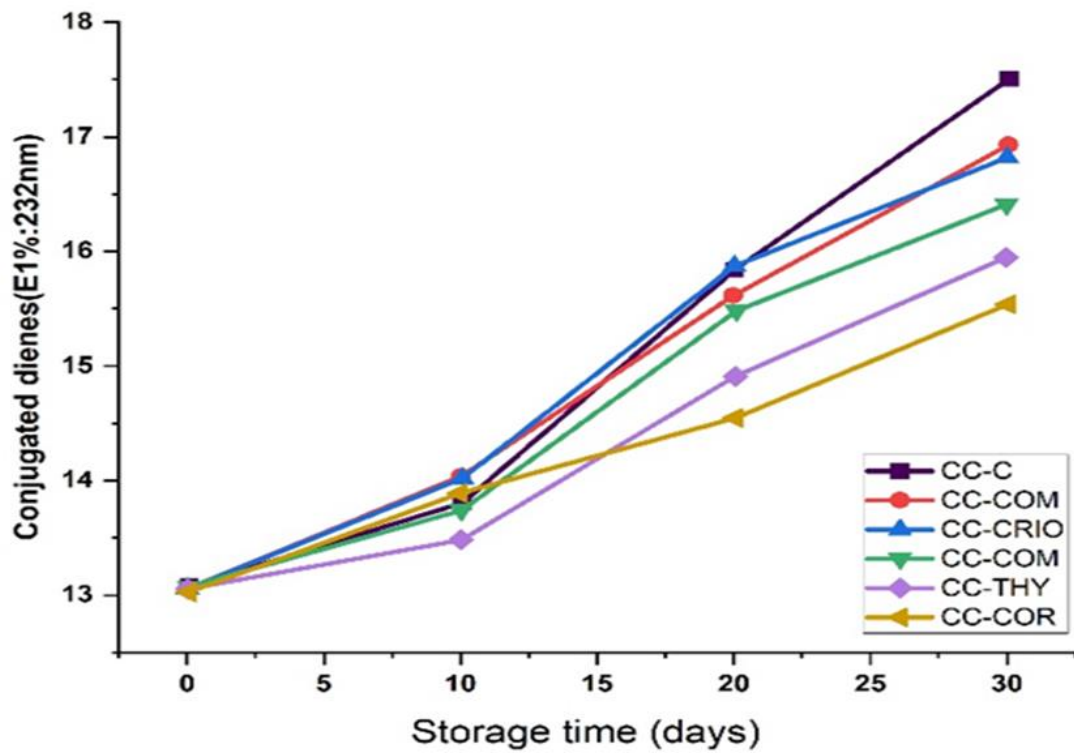


Figure 2: Graphical Diagram for Conjugated Dienes

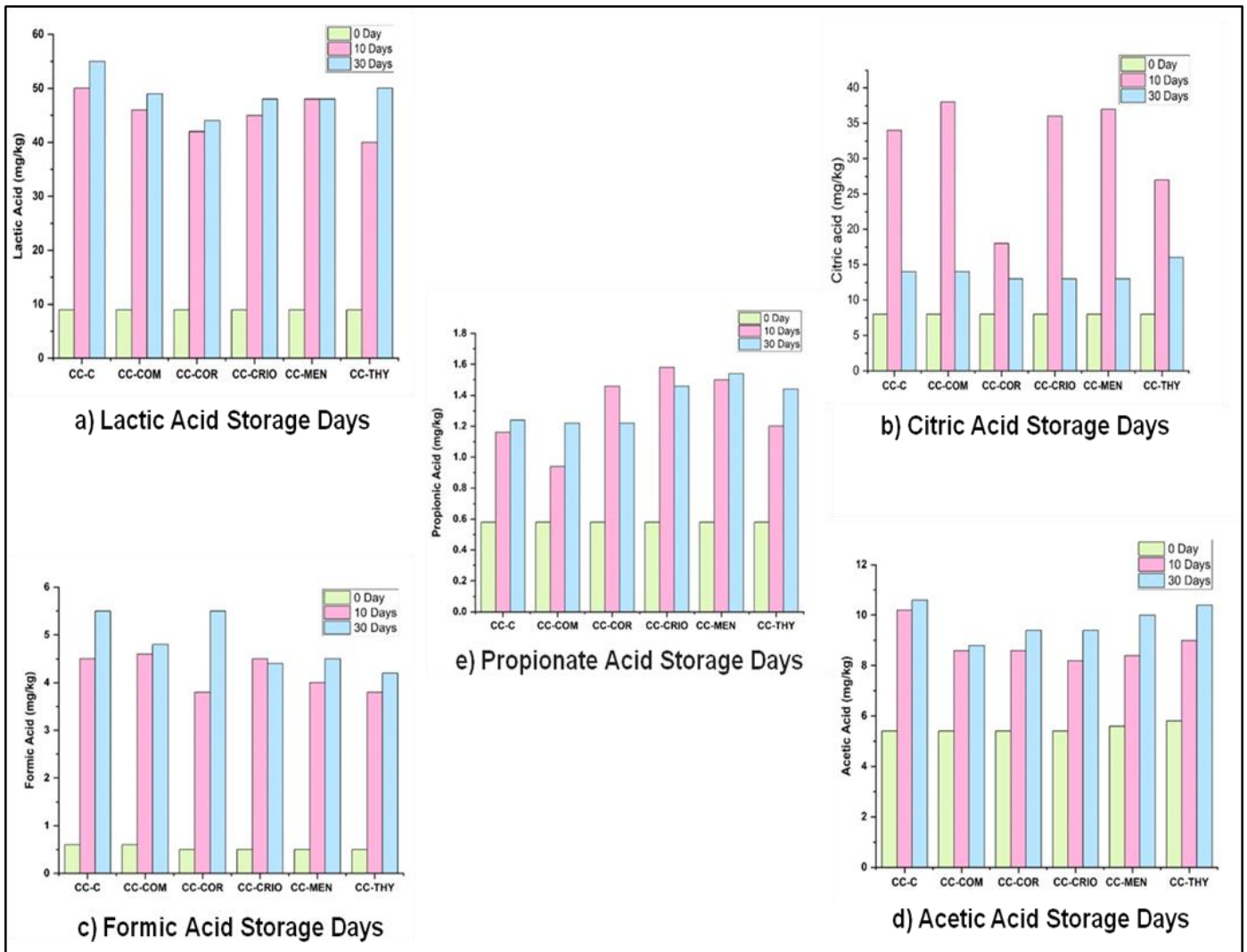


Figure 3: Result of significant variations in organic acid

having the greatest value (17.54). Because milk is a powerful antioxidant, several studies have shown that dairy products have high levels of absorption with regard to lipid peroxidation.

3.2 Organic Acid

Organic acids have an important role in imparting flavour and fragrance to most types of cheese. The lipolysis of milk fat produces acetic and butyric acids, whereas the regular biochemical metabolic of sheep produces citric, orotic, and uric acids, and bacteria produce formic, acetic, butyric, propionic, lactic, and pyruvic acids. Consequently, measuring organic acids quantitatively may be a useful way to track bacterial metabolic activity. Figure 3 displays the concentrations of OA in organic CC samples. Significant variations in organic acid concentration were observed after 30 days of storage.

3.2.1 Lactic Acid

The primary byproduct of lactose fermentation, lactic acid (Fig. 3a), peaked at 56.45 in CC-C and 42.5 mg and kg in CCCOR at storage's final. Cordobes, the final

therapy, was unlike the control cheese and the previous flavoured therapies. As a general rule, lactic acid concentrations were much greater than those of other organic acids.

3.2.2 Citric Acid

Intermediary metabolites such as citric and pyruvic acids exhibit erratic variations as cheese ages. With the help of certain moulds, glucose may ferment into citric acid. Additionally, the starting lactococci may employ citrate as a substrate to create acetic and pyruvic acids. After 10 days of storage, citric acid (Figure. 3b) was found at higher concentrations in the current research. At the conclusion of the storage, no appreciable variations across treatments were found for this organic acid.

3.2.3 Formic Acid

Pyruvate metabolism may be responsible for the high formic acid concentrations (Fig. 3c). Storage caused the formic acid concentration to dramatically rise. At the last storage day, the CC-C specimen had the maximum presence whereas the CC-THY sample had the least range.

3.2.4 Acetic Acid

A soury off-flavor might be transferred to cheese by detectable levels of acetic acid. Non-starter lactobacilli may also make formic anhydride from lactose or amino acids. After 10 storage days, big variation between treatments were found (Fig. 3d), with the control sample showing the highest value. At day 30 of storage, CC-C still had the largest acetic acid content (10.89 mg/kg). This finding suggests that these cheese samples had a greater level of heterofermentative metabolism. Additionally, after 30 days of storage, the “CC-COM, CC-COR, and CC-CRIO” specimen showed the least level of AA (Fig. 3d).

3.2.5 Propionate Acid

The development of propionate during storage in the samples of natural CC are depicts in figure 3e. Propionic acid concentrations were greater at the conclusion of storage than at the start of storage in all samples. This organic acid was present in smaller amounts in the CC-COM and CC-COR samples. At the conclusion of preservation, CC-MEN showed the greatest quantity of this OA (1.54 mg/kg), indicating that this EO had no preservation impact on natural CC. EOs, particularly those from the Cordobes and Compacto varieties, were shown to dramatically reduce organic acid generation while organic CC was being stored. It's possible that oregano EO's effect on microbial and enzyme activity contributes to the reduction in organic acid generation. After the CC had matured, the essential oil of oregano was added. Consequently, the EO has no effect on the ripening of a commodity or service, but this aggravate aids in the preservation of this product by, on the one hand, reducing the establishment of OA like acetic, lactic, and formic acids, primarily, and, in addition hand, lowering the pH in samples with OEO; where the concentration of acid compounds is related to the product's ripeness. Oregano EO-enhanced samples also show a diminished degradation of several fatty acids, including linolenic, elaidic, and a decreased rating of lipid oxidation markers such as HV and CD. All of these findings point to the essential oil of oregano's role as a preservative in this cheese. However, it would be wise to conduct a sensory analysis of the product to see how the inclusion of oregano essential oil affects its overall appeal to consumers.

4. Conclusions

Oregano essential oil slows the rate of chemical spoilage in organic CC. When Cordobes and Criollo essential oils (EOs) are added to this organic product, the longer polyunsaturated fatty acids are protected from oxidation processes. Organic cheese could benefit from the inclusion of essential oils of Cordobes or Compacto oregano in order to lower the organic acid formation linked with decreasing microbiological activity during storage. Manufacturers and customers alike would be pleased if this kind of natural substance were used as a food preservative. The essential oil of oregano (EO) may lengthen the distribution window for

perishable foods that might otherwise spoil quickly. Oregano essential oil (EO) is a natural, GRAS substance that can be grown and processed in organic environments, suggesting it may be beneficial as an organic preservative for organic food items.

Reference

- [1] S. Karunamay, S.R. Badhe, V. Shulka, and S. Jaiswal, (2020). Effect of essential oil of clove and oregano treated edible packaging film in extending the shelf life of paneer. *Pharm Inno J*, 9:pp.317-322.
- [2] O.S. Papadopoulou, A.A. Argyri, V.C. Bikouli, E. Lambrinea, and N. Chorianopoulos, (2022). Evaluating the Quality of Cheese Slices Packaged with Na-Alginate Edible Films Supplemented with Functional Lactic Acid Bacteria Cultures after High-Pressure Processing. *Foods*, 11(18):p.2855.
- [3] C.M. Asensio, N.R. Grosso, and H.R. Juliani, (2015). Quality preservation of organic cottage cheese using oregano essential oils. *LWT-Food Science and Technology*, 60(2):pp.664-671.
- [4] H.T. Diniz-Silva, L.R. Brandão, M. de Sousa Galvão, M.S. Madruga, J.F. Maciel, E.L. de Souza, and M. Magnani, (2020). Survival of *Lactobacillus acidophilus* LA-5 and *Escherichia coli* O157: H7 in Minas Frescal cheese made with oregano and rosemary essential oils. *Food microbiology*, 86:p.103348.
- [5] H.S. El-Sayed, and S.M. El-Sayed, (2021). A modern trend to preserve white soft cheese using nano-emulsified solutions containing cumin essential oil. *Environmental Nanotechnology, Monitoring & Management*, 16:p.100499.
- [6] J.V. Cama-Curasi, E. Saldaña, M.L. Cruzado-Bravo, C. Ambrosio, and J. Mayta-Hanco, (2022). Incorporation of nisin and oregano essential oil in cow's milk to improve the quality of fresh cheese. *Scientia Agropecuaria*, 13(4):pp.359-367.
- [7] L.I. Ahmed, N. Ibrahim, A.B. Abdel-Salam, and K.M. Fahim, (2021). Potential application of ginger, clove and thyme essential oils to improve soft cheese microbial safety and sensory characteristics. *Food Bioscience*, 42:p.101177.
- [8] H.T. Diniz-Silva, L.R. Brandão, M. de Sousa Galvão, M.S. Madruga, J.F. Maciel, E.L. de Souza, and M. Magnani, (2020). Survival of *Lactobacillus acidophilus* LA-5 and *Escherichia coli* O157: H7 in Minas Frescal cheese made with oregano and rosemary essential oils. *Food microbiology*, 86:p.103348.
- [9] M. Laranjo, A.M. Fernández-León, A.C. Agulheiro-Santos, M.E. Potes, and M. Elias, (2022). Essential oils of aromatic and medicinal

- plants play a role in food safety. *Journal of Food Processing and Preservation*, 46(8):p.e14278.
- [10] Z. Mahcene, A. Khelil, S. Hasni, F. Bozkurt, M.B. Goudjil, and F. Tornuk, (2021). Home-made cheese preservation using sodium alginate based on edible film incorporating essential oils. *Journal of Food Science and Technology*, 58:pp.2406-2419.
- [11] K. Saeed, I. Pasha, M.F. Jahangir Chughtai, Z. Ali, H. Bukhari, and M. Zuhair, (2022). Application of essential oils in food industry: challenges and innovation. *Journal of Essential Oil Research*, 34(2):pp.97-110.
- [12] J.S. Chacha, C.E. Ofoedu, and K. Xiao, (2022). Essential oil-based active polymer-based packaging system: A review of its effect on the antimicrobial, antioxidant, and sensory properties of beef and chicken meat. *Journal of Food Processing and Preservation*, 46(11):p.e16933.
- [13] A. Ramzy, (2023). Potential application of cumin, pepper and thyme spices on soft cheese quality. *Benha Veterinary Medical Journal*, 43(2):pp.97-103.
- [14] S. Rout, S. Tambe, R.K. Deshmukh, S. Mali, J. Cruz, P.P. Srivastav, P.D. Amin, K.K. Gaikwad, E.H. de Aguiar Andrade, and M.S. de Oliveira, (2022). Recent trends in the application of essential oils: The next generation of food preservation and food packaging. *Trends in Food Science & Technology*.
- [15] S.A. Sakr, M.I. ElShaer, A.L. Mohamed, and M.A. Bayoumi, (2022). Preservative Effect of Edible Chitosan Coated Liposomes Loaded with Natural Antimicrobial Agents in White Soft Cheese. *Journal of Advanced Veterinary Research*, 12(6):pp.651-657.