



Research Article

Effect of Garlic Extract (*Allium sativum*) as a Preservative in a Fermented Kefir Type Dairy Drink

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Abstract | Natural preservatives are widely used in the food industry since they have the ability to preserve or to be an antioxidant, but with the great difference that they are not harmful to health. Garlic with its compounds become a preservative agent, therefore, the objective of the research was to evaluate the effect of garlic extract (*Allium sativum*) as a preservative in a kefir-type fermented milk beverage. A completely randomized design was used where C (control) presented the percentages of natural kefir; T₁ 1Lt/ 3mlA; T₂ 1Lt/ 5mlA; T₃ 1Lt / 7mlA with three treatments and three replicates. Garlic extraction was carried out by a mechanical method which used a KM grater which consists of generating friction on a surface with sharp holes to separate the fibers into particles, colorimetry analysis was performed with a Konica Minolta CR-400 colorimeter, a viscosity analysis using a rotational viscometer and a hedonic scale of 7 points sensory analysis, the data were processed in the Minitab program, ANOVA and Dunnet. It was determined that there was a significant difference in the useful life of the product with respect to fungi, yeasts and mesophiles. In conclusion, results showed that the inclusion of 3ml, 5ml and 7ml of extract prolonged the shelf life without the presence of Coliforms and *E. coli*, reaching a durability of 21 days, however, at a higher concentration of garlic, acceptance decreased. The most widely accepted products in terms of color, odor, flavor, texture and general appearance parameters were treatments C (control) and T₂. It is concluded that the inclusion of garlic extract had an antimicrobial effect in the production of milk kefir.

Received | August 28, 2023; **Accepted** | April 17, 2024; **Published** | August 30, 2024

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Citation | Zambrano, P.V., L.V. Cortez, J.I. Arteaga, C.C. Ceballos and R.P. Alava. 2024. Effect of garlic extract (*Allium sativum*) as a preservative in a fermented kefir type dairy drink. *Sarhad Journal of Agriculture*, 39(Special issue 2): 80-92.

DOI | <https://dx.doi.org/10.17582/journal.sja/2023/39/s2.80.92>

Keywords | Natural preservative, Garlic extract, Kefir, Shelf life



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Introduction

Global bovine milk production between 2008 and 2018 grew at an average annual rate of 1.4 percent, and stood at an all-time high of 505.2

million tons so production of dairy products such as cheese, butter and milk powder, both skim and whole, also continues to grow (FIRA, 2019). In addition, the production and consumption of cow's milk has a primary importance in the national economy and

much of the world according to [Tapia \(2020\)](#), since it is a relevant component in people's diet, especially for its content of high-value proteins that contribute to an adequate diet, especially in childhood. In developed countries, the consumption of fresh dairy products was indicated without major variations, while processed products could grow by around 1% per year in their consumption, since international trade in dairy products represents a small fraction of production, so much of the consumption is made in the local markets of each country.

Dairy products such as milk and its fermented derivatives are known to be perishable foods ([Reyna and Arteaga, 2022](#)), so after obtaining them, it is necessary to apply strict quality control and surveillance in order to efficiently guarantee the cold chain until they reach the different households ([Uscanga et al., 2019](#); [Aguirre et al., 2023](#)).

Preservatives are used in the food industry to maintain the sanitary quality of food, extend the shelf life of the product and limit the growth of pathogenic microorganisms ([Enríquez et al., 2023](#)). Considering that for their use they must comply with characteristics that limit the resistance of harmful microorganisms, without affecting useful microorganisms ([Mariani et al., 2021](#)).

According to their origin we can separate the additives in to 2 groups, the natural ones, which are extracted directly from nature, from some vegetables, minerals and animals and those that do not exist in nature and must be synthesized or bio synthesized artificially ([Burgos, 2020](#)).

The development of functional foods has been an opportunity to improve the quality of the diet and the optimal selection of foods that positively affect the health and well-being of the individual. Thus, different research has shown us that fermented dairy products represent an excellent source for a range of probiotics that give a positive value to milk ([Taco and García, 2021](#)).

From this aspect [Basurto and Franco \(2019\)](#), describe the inhibitory action of garlic extract on the growth of several bacterial genera, which act directly on inhibition of enzymes used in the metabolism of microorganisms, delay in the process of protein and nucleic acid synthesis, and deterioration of the cell

membrane ([Grajales et al., 2021](#)).

In the case of fermented dairy products, it is intended to achieve better preservation with the use of a natural preservative from garlic extract, which in addition to not being harmful to health will help facilitate the conservation of this type of products, since the objective of this research is to evaluate the effect of garlic extract (*Allium sativum*) as a preservative in a fermented dairy drink type kefir.

Materials and Methods

Material experimental

The selected dairy product corresponded to a fermented milk called Kefir, based on the growing diffusion of dairy products and the high benefit of consumption of this drink ([Campaña and Aguilar, 2021](#)).

Raw material collection

The dairy raw materials used to obtain kefir was previously pasteurized raw milk, acquired from the "Santa Martha" farm, located in the Camareta site of the Chone canton, Manabí province, which was valued according to the requirements established by the [NTE INEN 2395 \(2011\)](#); the kefir grains were purchased at the "KEIF ORGANICS" in the city of Quito.

The non-dairy raw material used was the garlic extract (*Allium sativum*) obtained through the application of a mechanical method which consists of generating friction on a surface with sharp holes to separate the garlic fibers into particles and obtain a higher yield of the extract and by means of a nylon mesh a pressure will be applied to obtain the liquid and not lose its natural consistency, This procedure was performed in the Laboratory of Microbiology, Bromatology, Chemistry and Biology and in the laboratory of Dairy Faculty of Zootechnical Sciences, garlic was acquired in the local market of the Canton Chone with an index of medium maturity and large teeth.

Experimental design

In the research, an experimental design was used in completely random blocks with a total of three replicas for each of the treatments. A total of four treatments were used, including three concentrations of garlic extract (3, 5 and 7 ml/l of kefir), plus a control treatment (no garlic extract) ([Table 1](#)). The

preparation of the kefir formulations were developed according to the concentrations described in Table 2.

Table 1: Arrangement of treatments.

Treatments	Codes	Formulations	Replicas
1	Control	0 mL garlic extract/litre kefir	3
2	T ₁	3 mL garlic extract/litre kefir	3
3	T ₂	5 mL garlic extract/litre kefir	3
4	T ₃	7 mL garlic extract/litre kefir	3

Table 2: Formulation of kefir with the different concentrations of garlic extract.

Inputs	Unit	C (Control)	T1	T2	T3
Bovine Milk	Lt	1	1	1	1
Kefir granules	g	6	6	6	6
Garlic extract	ml	0	3	5	7

Description of the process of obtaining garlic extract

- Reception of raw material: A garlic with an intermediate maturity of large cloves and that meets safety standards is received.
- Washing and peeling: Proceed to wash the bark of the garlic and proceed with a peel to obtain the clove.
- Weighing: We weigh the total tooth that we are going to use in the process, which was a total of 450g.
- Grating: A special ray was used for garlic KM brand, which allows to grate the garlic in an innocuous way.
- Filtering: Once the garlic clove is grated, it is filtered into a nylon mesh which allowed the liquid to be extracted from the garlic.
- Packaging: Packed in sterilized glass jar.
- Stored: Stored at a temperature of 4°C.

Description of the kefir production process

- Reception of the raw material: The raw material and inputs to be used are received and it is verified that they comply with the quality standards.
- Milk filtering: Milk is filtered in order to remove impurities that could be found in the product.
- Input weighing: The described inputs are weighed according to the formulations established for the product.
- Pasteurization: The milk is previously pasteurized at a temperature of 80°C for a period of time of 15 min.
- Inoculation: The inoculation of kefir is carried out according to the quantities required in the

formulation.

- Incubation: Incubation is carried out for a period of time of 24 hours, at room temperature (23-28 °C).
- Filtering: A filtering is carried out in order to eliminate possible residues of the kefir nodules.
- Addition of garlic extract: Prior to inclusion, the extracts are obtained and added in different concentrations.
- Packaging: Packaged at room temperature in compliance with safety requirements.
- Storage: Stored at a temperature of 4 °C.

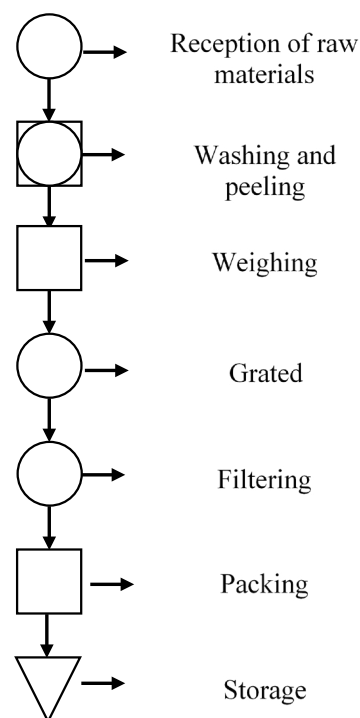


Figure 1: Flowchart to make garlic extract (*Allium sativum*).

Characterization of garlic extract

The physicochemical characterisation of the garlic extracts was carried out according to the criteria outlined by Velázquez (2023). An assessment of the pH of the garlic extract was carried out using a digital refractometer, for this the sample was placed in a beaker with a capacity of 50 ml and then the respective measurement was made, the same as that was made to three samples. The acidity assessment was performed, applying the potentiometric titration with NaOH 0.1 N up to an established pH point and the results were expressed as a percentage of pyruvic acid, using a conversion factor of 0.11. To determine the phenolic content was calculated using the Folin-Ciocalteu method, which the absorbance of the samples was measured at 760 nm in a microplate reader (Johnson et al., 2022).

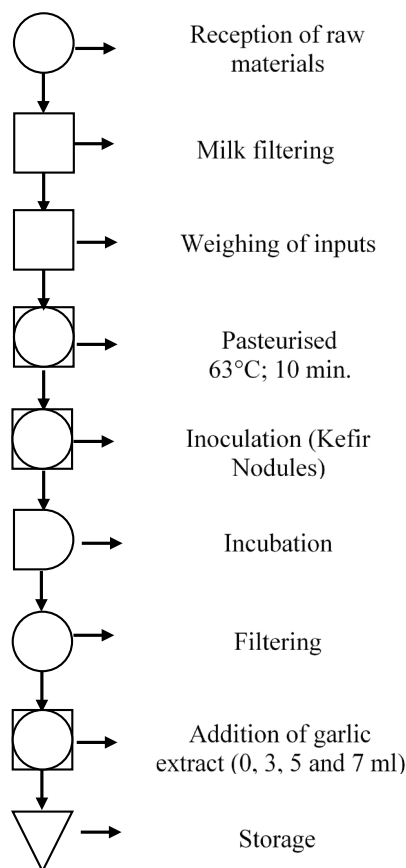


Figure 2: Flowchart to make a fermented kefir-type milk drink with the addition of garlic extract (*Allium sativum*).

Phytochemical screening

A qualitative photochemical screening of the garlic extract was carried out in which the following parameters were evaluated:

Phenols

The determination of Phenols will be done by placing 1 ml of the extract and six drops of sodium hydroxide (NaOH) at 10% m / v. If a blue color is observed, the sample is positive (Rivas *et al.*, 2020).

Flavonoids

The presence of flavonoids was affected by the Shinoda method. 1 mL of the extract shall be used and a magnesium shaving with 1 ml of concentrated hydrochloric acid (HCl) shall be placed. After five minutes of the reaction, 1 mL of isoamyl alcohol will be placed. It is considered positive when isoamyl alcohol is colored carmelite red (+ little presence), carmelite orange (++ medium presence) and intense carmelite green (+++ high presence) (Rivas *et al.*, 2020).

Tannins

The determination of tannins was carried out

qualitatively; for this purpose, a test tube shall be used in which a test tube with 1 mL of the extract and three drops of ferric chloride at concentrations of 5% m/v shall be placed and the change in colouration is expected. It tests positive when the reaction should have a wine-red, blue, or greenish-yellow color (Rivas *et al.*, 2020).

Saponina

The use of a test tube in which one (1) mL of the extract was placed and five (5) mL of distilled water was manually stirred for 10 minutes was qualitatively determined. The expected result is the formation of foam, if it is maintained for two (2) minutes the result is positive (Rivas *et al.*, 2020).

Alkaloids

In a test tube one (1) mL of extract and three (3) drops of ferric chloride (FeCl₃) were placed in concentrations of 5% m / v, the change of coloration was expected immediately, for the reaction to be positive it must present a wine red, blue or greenish yellow color (Rivas *et al.*, 2020).

Reducing sugars

Reducing sugars shall be determined by the Benndict method. 0.5 mL of extract was placed, the Benndict reagent was added until a blue coloration was obtained and it was taken to a water bath for 10 minutes. It is rated as follows +: There was a positive reaction/ reaction -: There was no reaction/negative reaction (Rivas *et al.*, 2020).

Catequinas

A drop of the fractions of each of the extracts was taken and placed on filter paper. A solution of calcium carbonate (CaCO₃) was applied to the stain. The appearance of a Carmelite green spot in UV light indicates that it is a positive test (Terán, 2023).

Cardiotónicos

By means of a Kedde test, the same one that allows to recognize an alcoholic extract the presence of cardiotonic glycosides. An aliquot of the extract is added in ethanol mixed with 1mL of reagent and left to stand for 10 minutes. In a positive test, persistent violet coloration develops for one to two hours (Rodríguez *et al.*, 2021).

Behavior of the shelf life during the storage of kefir

The shelf life behavior of the treatments under study

was evaluated in order to analyze the efficacy of garlic extract in the microbiological preservation of kefir. In each treatment, evaluations of the microbiological composition were carried out where the presence of total coliforms was evaluated by the ISO 4832: 2006/ Compact Dry test method; the determination of *Escherichia coli* by the method of NTE INEN 1529-8/ Compact Dry; Fungi and yeasts by the test method of NTE INEN 1529-10: 2013/ Compact Dry, and *Mesophilic Aerobes* by the method NTE INEN 1529-5: 2006/ Compact Dry. The evaluations were carried out at a time interval of seven days up to 21 days (0, 7, 14 and 21 days).

Sensory evaluation

A sensory evaluation of each of the treatments under study was carried out, where the parameters taste, smell, color, texture and general appearance were evaluated. A hedonic test was used with an evaluation scale from 1 to 7, with one being the lowest grade and seven the highest. For this, a total of 15 semi-trained tasters were used among students, teachers and people who consume dairy products on a regular basis, who were previously trained on the details to be assessed in each of the formulations.

Viscosity assessment

Viscosity was assessed using a digital rotational viscometer meter Fluidimeter 1-1000000cPs NDJ-79A. It began by placing the samples (three replicas for each treatment), in a glass container with a capacity of 300 mL, which were transferred to the measuring equipment. Each of the measurements was made at a temperature of 28°C. Measurements were expressed in cPs.

Colorimetric evaluation of the treatments under study

The color evaluation was carried out using a Konica Minolta CR-400 colorimeter using the CieLab space where the parameters L*=luminosity, a*= red/green coordinates (+a indicates red, -a indicates green) and b* = yellow/blue coordinates (+b indicates yellow, -b indicates blue) were considered.

For the calculation of the difference of the Deltas was made by using the following formula:

$$\Delta E = (L1-L2)^2 + (a1-a2)^2 + (b1-b2)^2$$

After that a comparison was made between the samples and the acceptance of the samples was determined by

the standards establishing the following parameters:

0-1 excellent; 1-2 good; 2-4 normal; 4-5 sufficient and higher than 5 bad.

Economic valuation

- To make an economic valuation, the following elements were used:
- Cost of Raw Materials
- Economic comparison of production between the experimental product and the control.

Statistical analysis

The statistical analyses were developed using the Minitab statistical software. ANOVA and DUNNET tests were carried out. These analyses were applied in the microbiological evaluation of the treatments under study. The results of the sensory panel were performed by applying kruskal Wallis tests.

Results and Discussion

Characterization of garlic extract

The physicochemical characterization of the garlic extract was carried out, resulting in the following:

As shown in Table 3, the physicochemical analyses of garlic extract showed a phenolic content of 1.3601 ± 0.05 , pH of 6.26 ± 0.15 and acidity content of 0.74 ± 0.01 . Research by Velázquez (2021) documents a pH content of 6.8 ± 0.2 , the results they obtained are close to those of this research since they worked with the same maturity.

Table 3: Physicochemical parameters of garlic extract.

Physico-chemical	Average $\pm$ D.E	Unit	Method
Phenolic content	$1,3601 \pm 0,05$	mg lalic acid/g	Folin-Ciocalteu
pH	$6,26 \pm 0,15$	--	AOAC-981.12
Acidity	$0,74 \pm 0,01$	%	NTE INEN 13

According to the results presented by Martínez *et al.*, (2021), when characterization of the physicochemical properties of garlic extract from plants of 'Criollos' clones which is described as a result pH ranges of 6.50 to 6.59 and an acidity of 0.32 to 0.37, this was done since the raw material is grown and harvested in different ways with the help of a liquid bioproduct, which causes a different variation of its pH and acidity, however in the pH and in the acidity they do

not present much similarity to our results.

The presence of phenols in the garlic extract was $1,3601 \pm 0,05$ mg lalic acid/g. However, [Bar et al. \(2022\)](#) indicate that there may be variations in the presence of phenols associated with external factors such as climatic conditions, nutritional richness of soils, and time of harvest of the raw material.

Characterization of secondary metabolites

Table 4 shows the characterization of the assay used in garlic extracts, a greater presence of phenols was obtained, followed by a moderate presence of flavonoids, tannins, saponins, alkaloids, reducing and cardiogenic sugars. On the other hand, the determination of catechins was absent.

Table 4: *Phytochemical screening of garlic aqueous extract.*

Metabolites	Practice	Value
Phenols	Folin	++
Flavonoids	Shinoda	+
Tannins	Ferric chloride	+
Saponins	Foam	+
Alkaloids	Wagner	+
Reducing sugars	Fehling	+
Catequinas	Sodium carbonate	-
Cardiotónicos	Kedde	+

The presence of secondary metabolites in garlic extract coincides with the results reported by [González and](#)

[Farroñan \(2023\)](#), where they document the presence of tannins, flavonoids, phenolic compounds and alkaloids. According to [Quesada \(2020\)](#), the presence of these secondary metabolites has been related to higher antioxidant and antimicrobial activity, which makes it positive for this research study.

In the same sense, [Hernández and Rubio \(2017\)](#), when analyzing the presence of secondary metabolites in garlic extract document as results the presence of triterpenoids and phenolic compounds, while documenting the absence of alkaloids and flavonoids, differing from the results exposed in the present study.

Shelf life microbiological parameters in kefir with garlic extract

Microbiology evaluation results of total coliforms in kefir with garlic extract during days 0, 7, 14 and 21. The analysis of the shelf life of kefir with the three concentrations of garlic versus a control treatment shows that the presence of coliforms (**Table 5**) was presenting variations between each of the weeks in which the research was developed.

During the first week in which the research was developed, it was obtained that the treatments showed the same behavior in each of the means, unlike the second week where the control treatment presented statistical differences with the other treatments, being in turn the one with the highest content of Coliforms $2.08 \times 10^2 \pm 0.07a$.

Table 5: *Analysis of total coliforms.*

Total coliforms CFU/mL				
Treatments	Day 0 No difference (sd)	Day 7 Average $\pm$ D. E	Day 14 Average $\pm$ D. E	Day 21 Average $\pm$ D. E
C (Control)	0 UFC/MI	$2,08 \times 10^2 \pm 0,07^a$	$1,06 \times 10^2 \pm 0,07^a$	$1,02 \times 10^2 \pm 0,08^a$
T1	0 UFC/mL	$0,00 \pm 0,00$	$0,00 \pm 0,00$	$0,00 \pm 0,00$
T2	0 UFC/mL	$0,00 \pm 0,00$	$0,00 \pm 0,00$	$0,00 \pm 0,00$
T3	0 UFC/mL	$0,00 \pm 0,00$	$0,00 \pm 0,00$	$0,00 \pm 0,00$
p-value	Sd	0,000**	0,000**	0,000**

Table 6: *E. coli análisis.*

Escherichia coli				
Treatments	Day 0 No difference (sd)	Day 7 Average $\pm$ D. E	Day 14 Average $\pm$ D. E	Day 21 Average $\pm$ D. E
C (Control)	Absence	Absence	Absence	Absence
T1	Absence	Absence	Absence	Absence
T2	Absence	Absence	Absence	Absence
T3	Absence	Absence	Absence	Absence

According to the results obtained up to 21 days, the results show that treatment C (control) is within the maximum permissible index to identify an acceptable level of quality of the NTE INEN 2395 (2011). In the case of the T1, T2 and T3 treatments, the durability of the characteristics in terms of this parameter was maintained up to 21 days, showing as a result the inhibitory effect of garlic extract against the presence of total coliforms UFC/mL.

Flores (2019), when developing a fermented kefir-type milk drink from acid whey and milk, mesophilic aerobes 30×10^3 cfu/g and coliform numbering 23 NMP/g, which are superior to the results documented in the research in reference to coliform content.

On the other hand, studies carried out by Benavides (2021), when evaluating the shelf life of a kefir, obtained as a result a content <10 CFU/g with respect to the presence of total coliforms up to 30 days after the research.

Evaluation results microbiology of E. coli in kefir with garlic extract during days 0, 7, 14 and 21

Escherichia coli showed that during the 21 days in which the research was carried out, the absence of this type of microorganism in each of the treatments under study was presented.

Preliminary research developed by de Oliveira (2023), documents that the presence of kefir granules can exert an important effect in the reduction of pathogens, including the presence of *Escherichia coli*, and this effect is also reflected in the decrease of Salmonella Typhimurium proliferation levels (Medrano et al., 2020).

These criteria agree with those presented by Abraham (2019), where he describes that the absence of this type of microorganisms is given by the probiotic properties attributed to microorganisms isolated from

kefir, which have the ability to antagonize the possible effects given by the development of *Escherichia coli*.

Despite no reports on the inclusion of *Allium sativum* garlic in the production of this type of beverage, the antimicrobial effect of this type of extract on cultures of microorganisms such as cultures of *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Panchi, 2016; Machado, 2023). It is evident that carefully made kefir shows absence of harmful microorganisms and in this case it has been shown that with the inclusion of garlic extract to the product increases the inhibition of bacterial growth thus better reinforcing the product.

Results of microbiology evaluation of fungi and yeasts in kefir with garlic extract during days 0, 7, 14 and 21

The presence of fungi and yeasts from each of the treatments under study showed differences in this type of microorganisms. At the beginning of the research (Day 0), T₁ and C (control) presented a greater amount of fungi and yeasts unlike T₂ and T₃ presented a lower amount due to the difference in addition of ml/garlic. At the end of the mean control days between day 7 and 14 there was no significant difference between the treatments that included garlic extract concentrations. At day 21 it was obtained that the treatments T₂ $9.06 \times 10^3 \pm 0.05$ and T₃ $4.01 \times 10^3 \pm 0.01$ were the ones that obtained the best performance according to the INEN 2395 standard that establishes a maximum of 10×4 and the C (control) was the only one that showed deterioration at 21 days.

Studies carried out by Chicaiza (2018), with regard to molds and yeasts, their presence is justified by the INEN 2395 fermented milks standard that establishes the minimum range of 10^4 UFC/g of kefir presence to define the substrate as a probiotic; therefore it is affirmed that the result obtained is favorable due to the high requirement of the culture.

Table 7: Analysis of fungi and yeasts.

Treatments	Fungi and yeasts UP/mL			
	Day 0 No difference (sd)	Day 7 average $\pm$ D. E	Day 14 average $\pm$ D. E	Day 21 average $\pm$ D. E
C (Control)	$4,05 \times 10^2 \pm 0,05^a$	$1,38 \times 10^3 \pm 0,06^a$	$1,90 \times 10^3 \pm 0,01^a$	$1,30 \times 10^4 \pm 0,01^a$
T1	$4,02 \times 10^2 \pm 0,02^a$	$1,30 \times 10^3 \pm 0,02$	$1,81 \times 10^3 \pm 0,05$	$1,00 \times 10^4 \pm 0,01$
T2	$3,41 \times 10^2 \pm 0,02$	$1,10 \times 10^3 \pm 0,02$	$1,55 \times 10^3 \pm 0,04$	$9,06 \times 10^3 \pm 0,05$
T3	$3,32 \times 10^2 \pm 0,01$	$1,00 \times 10^3 \pm 0,01$	$1,41 \times 10^3 \pm 0,01$	$4,01 \times 10^3 \pm 0,01$
p-valor	0,000**	0,000**	0,000**	0,000**

Benavides (2021), when studying the shelf life of a kefir with the inclusion of vegetable raw materials (carrot, spinach), describes as a result a content of fungi and yeasts less than 10 CFUs/g, being lower than those described in the research (Table 7).

As for the use of plant extracts of plant origin as alternatives that allow the conservation of the same during shelf life, however, according to the results described in the research it is described that the benefits of garlic extract allowed a higher concentration to reach a shelf life of 21 days.

Results of microbiology evaluation of mesophilic aerobes in kefir with garlic extract during days 0, 7, 14 and 21

According to the results obtained, it is described that the results of the mesophilic aerobes CFU/mL for each of the treatments under study was obtained that during each of the weeks in which the presence of this type of microorganisms was evaluated, significant differences were found between the control treatment with the other treatments. In this case, it can be seen that the treatments that included the different concentrations of garlic extract presented a lower mesophilic aerobic content CFU/mL, with 21 values between 3.32×10^5 to 3.55×10^5 CFU/mL during the day compared to the control treatment with 3.93×10^5 .

Flores (2019), when evaluating the microbiological characteristics of the fermented kefir type drink obtained as a result a numbering of mesophilic aerobes of 30×10^3 cfu/g, therefore, the product is

in acceptable conditions and suitable for human consumption.

In general, the importance of the use of natural preservatives in the food industry is highlighted as an alternative that can replace the use of synthetic preservatives, maintaining the characteristics of the product for a long period of time (Batista *et al.*, 2022; Lozano *et al.*, 2022), which is also complemented by an improvement in food safety without compromising the final quality of the product, which in turn reduces the presence of adverse effects on the health of consumers (Ojeda *et al.*, 2022).

Sensory analysis and measuring instrument

The results of the sensory evaluation of kefir with the three concentrations of garlic extract (Table 9), resulted in significant differences between the parameters taste, smell, color, texture and general appearance which is graphed in the Annex (9), in this case it is described that the control treatment showed similarity between the average values of the treatments studied with respect to each of the sensory parameters, which in turn are shown to be superior among the rating ranges. Studies carried out by Alvarado (2018), when carrying out a sensory evaluation of a drink made from whey powder and kefir granules found significant differences between the parameters smell, taste and general acceptance of finding scores of 6 to 7, these being higher than those described in the research.

Table 8: *Analysis of mesophilic aerobes.*

Treatments	Mesophilic aerobics CFU/mL			
	DAY 0 average $\pm$ D. E	Day 7 average $\pm$ D. E	Day 14 average $\pm$ D. E	Day 21 average $\pm$ D. E
C (Control)	$3,17 \times 10^4 \pm 0,02^a$	$7,29 \times 10^4 \pm 0,01^a$	$2,02 \times 10^5 \pm 0,03^a$	$3,93 \times 10^5 \pm 0,03^a$
T1	$2,56 \times 10^4 \pm 0,05$	$7,05 \times 10^4 \pm 0,04$	$1,17 \times 10^5 \pm 0,02$	$3,55 \times 10^5 \pm 0,05$
T2	$2,42 \times 10^4 \pm 0,02$	$5,76 \times 10^4 \pm 0,04$	$1,55 \times 10^5 \pm 0,05$	$3,32 \times 10^5 \pm 0,03$
T3	$2,76 \times 10^4 \pm 0,04$	$6,43 \times 10^4 \pm 0,05$	$1,32 \times 10^5 \pm 0,03$	$3,42 \times 10^5 \pm 0,02$

Table 9: *Sensory analysis of the fermented milk drink type kefir.*

Treatment	Taste	Smell	Color	Texture	Apa_Gen
C (control)	$6,60 \pm 0,50$ b	$6,26 \pm 0,46$ b	$6,00 \pm 1,00$ b	$5,93 \pm 1,03$ c	$6,46 \pm 0,56$ a
T1	$5,20 \pm 1,17$ a	$4,67 \pm 1,19$ a	$4,6 \pm 1,58$ a	$4,00 \pm 1,59$ a	$4,67 \pm 0,87$ b
T2	$6,27 \pm 0,77$ b	$6,13 \pm 0,72$ b	$5,93 \pm 1,0$ b	$5,80 \pm 0,11$ bc	$6,13 \pm 0,72$ a
T3	$5,07 \pm 1,24$ a	$4,8 \pm 1,22$ a	$4,87 \pm 1,09$ a	$4,73 \pm 1,48$ ab	$5,2 \pm 0,91$ b
p-valor	0,0005	0,0001	0,0077	0,0020	0,0001

While, [Rhodes \(2019\)](#), in the elaboration of a kefir based on with different proportions of kefir describes scores of 3 to 5 in the parameters color, smell, taste and texture, specifying that this type of products is influenced by the ingredients (milk, sugar, kefir granules) as well as the parameters used in the production of the drink have their own effect on the sensory characteristics of the final product.

On the other hand, [Flores \(2019\)](#), when carrying out a study on the concentrations of milk and whey in the elaboration of a kefir obtained as a result a better acceptance documenta scores of 4.5 to 5.5 in the sensory parameters color, smell and taste.

Table 10 shows the results of the viscosity of kefir with the inclusion of the three concentrations of garlic extract showed statistical differences between the treatments under study, In this case it was obtained that the highest viscosity was found in the T2 treatment with a mean of 1096.90 ± 2.13 , while lower viscosity was found in the T1 treatment with a total of $1056.83 \pm 1, 27$. In this case it is described that the behavior with respect to viscosity showed a similar behavior between the formulations that included the garlic extract, the statistical data are in the Annex (10).

Table 10: *Physicochemical parameter of viscosity in kefir with garlic extract.*

Treatments	Viscosity average $\pm$ D. E	Unit	Method
C (Control)	1061,77 $\pm$ 3,94	mPa.s (cP)	Instrumental rotational viscometer
T1	1056,83 $\pm$ 1,27		
T2	1096,90 $\pm$ 2,13		
T3	1092,07 $\pm$ 4,31		
p-valor	0,000**		

[Pretell and Urraca \(2016\)](#), when carrying out the rheological characterization of a kefir type drink from cow's milk and goat's milk, documents as a result a viscosity of 281.83 mPa.s and 299.00 mPa.s respectively in each of the raw materials used, these results are lower than those documented in the research.

For their part, [Gutierrez et al. \(2019\)](#), when studying the different concentrations of inoculum and incubation time, found statistical differences between the treatments studied, being in this case the inclusion

of 3ml of the inoculum which presented a greater viscosity at 24 hours of fermentation of 3200 cPs.

Regarding the differences in color through the CieLab space (**Table 11**), they showed as a result that the luminosity was similar between the control treatment with the treatment that included the concentrations of garlic extract, this because garlic extract contains carotenoids that can influence the colorimetric properties of this type of beverages ([Otunola et al., 2010](#)).

Table 11: *Color parameter in kefir with garlic extract.*

Treatments	L	A	B	ΔE	Method
C (Control)	16,94	0,33	1,48	0,85	Instrumentation colourimeter
T1	16,5	-0,27	1,30	1,35	
T2	16,91	-0,77	1,46	0,42	
T3	16,21	-0,16	0,89	1,43	

In the case of the coordinate a* the results show that differences between the T3 treatment with the other treatments, with lower values -0.16. In the case of treatments that included garlic extract, they presented a slight tendency to be within the coordinates that determine green chromaticity. On the other hand, the coordinates of side b* showed similarity between the control treatment with the treatments T1 - T2 unlike with the T3 where a lower fixation of the coloration in the yellow chromatic coordinate was observed.

The colour of kefir is influenced by the natural properties of the ingredients used in its production, emphasising that kefir granules do not affect the final colour of the product at all ([Bengoa, 2020](#)).

Coinciding with what is described by [Ferrari et al. \(2020\)](#), where it describes that the colorations of kefir are white or slightly yellowish, being in similarity with the results obtained in the research where white colors and a slight tendency to yellowish are denoted.

The results of the calculation of ΔE show as a result that as the concentration of the garlic extract increased, the results were approaching the level of excellent quality (0-1), considering the low contribution of pigments such as carotenoids present in the garlic extract which in turn is linked to the characteristics of the drink. In this case it is observed that compared to the control treatment the comparisons of delta E show a greater similarity with the T2 treatment.

Economic valuation

Table 12 shows the costs corresponding to the formulation of kefir made with the application of the natural preservative from garlic and based on a commercial product of the dairy industry Nestlé.

This formulation is calculated for the T2 treatment of 5 ml of garlic since it was the most accepted by the panelists. According to Álvarez (2021), the price of Nestlé kefir has a value of 1.69 euros which is equivalent to approximately 1.80 dollars in a presentation of 150g and is only being marketed in European countries. Making a relationship with our product that has a production value of 4 dollars per 1000 grams by placing them in containers of 100 g at a value of \$ 1 gives a total of \$ 10 net, where the estimated profit is \$ 6 per liter in production taking into account that the kefir granules can be reused for more products which causes to be a social and economic impact for being a product nutritional as it is kefir and the ease of obtaining garlic and applying it as a natural preservative in turn allowing the product to obtain an additional added value than it has. Díaz et al., (2020), studied the feasibility for the creation of a plant for the production of milk kefir resulted in a cost of 16.10 soles (Peru) equivalent to a total of approximately \$4.28.

Table 12: Economic evaluation of the best treatment in T2 study (1000g).

Inputs	g	Costs (USD)	%
Milk	1000	0,5	12,5
Kefir	6	3	75
Preservative (Garlic)	60	0.5	12,5
Total		4	100

Conclusions and Recommendations

- It was determined that in the secondary metabolites of garlic extract (*Allium sativum*) showed a low presence of saponins, alkaloids, reducing sugars, cardiogenic and a regular presence of phenols, indicating based on the results that the use of this product thanks to the compounds present is useful in the preservation of food and allows to reduce the use of artificial preservatives.
- It was satisfactorily achieved that the inclusion of 3ml, 5ml and 7 ml of garlic extract will prolong the shelf life of the product showing the absence of total coliforms unlike the control treatment

and as for the analysis of *E. coli* all treatments obtained the same behavior of the absence of this, reaching a durability of 21 days maintaining the quality of the product.

- It was obtained that the product with the highest acceptance in terms of sensory parameters were the control and T2 treatments (5ml of garlic extract), where ratings higher than 5.80 were found, establishing that the addition of 5 ml of garlic is the most indicated for the conservation of the dairy product type kefir tested both in sensory analysis, instrumental and microbiological.

Acknowledgement

Authors would like to express their gratitude to Dios and the Technical University of Manabí, as well as each of the authors of this research.

Novelty Statement

This study uniquely explores the application of garlic (*Allium sativum*) extract as a natural preservative in a fermented kefir-type dairy drink, a concept that has not been extensively investigated in the field of dairy preservation. While garlic's antimicrobial properties are well-documented, its integration into kefir, a probiotic-rich dairy product, introduces a novel approach to enhancing the shelf life and safety of such beverages without compromising their beneficial microbial content. The findings provide new insights into the dual role of garlic extract as both a preservative and a potential enhancer of the probiotic qualities in fermented dairy drinks, offering a natural alternative to synthetic preservatives.

Author's Contribution

Plinio Vargas Zambrano: Designed the study, performed the field work and collected the data, analyzed the data and drafted the manuscript, conducted the research, prepared and drafted the initial manuscript. Conceptualization, methodology, visualization, resources, and writing— original draft preparation.

Luis Vásquez Cortez: Designed the study, performed the field work and collected the data, analyzed the data and drafted the manuscript, conducted the research, prepared and drafted the initial manuscript. Conceptualization, methodology, visualization, resources, and writing— original draft preparation.

Julio Ibarra Arteaga: Designed the study, performed the field work and collected the data, analyzed the data and drafted the manuscript. Conducted the research, prepared and drafted the initial manuscript.

Camilo Chávez Ceballos: Conceptualization, data curation, and writing—review & editing.

Ramona Párraga Alava: Designed the study, analyzed the data and drafted the manuscript. Conceptualization, data curation, and writing—review & editing.

Conflict of interest

The authors have declared no conflict of interest.

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