



## STUDY OF THE KINETICS OF MILK KEFIR USING SOY GERM AS SUBSTRATE

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### SUMMARY

The main objective of this study was to analyze the growth kinetics of milk kefir on bean sprouts (*Glycine max*) as a germinated substrate for 4 days by the glass jar method followed by extracts. Ratio 1:3 (soy: water). 3% kefir grains (activated) inoculated with soluble bud extracts. The soluble extract of bean sprouts has a total sugar content of 5.57% and a fermentable carbohydrate of 3.37 g / L (sucrose), which is a source of nutrition and energy reserves; 1.40% of lipids are used for catabolic grain activity, while protein increases the amount of soluble nitrogen by 6.66%. The pH is 6.67 and the acidity is 0.17%, inversely proportional to lactic acid. The number of microbial populations of soluble extracts of kefirada sprouts reached the logarithmic stage of development after 24 hours for lactic acid bacteria and 48 hours for acetic acid bacteria and yeast, consuming the nutrients of the beverage, influencing its metabolites on the pH of the medium; The period of decomposition of lactic acid bacteria, acetic acid and yeast is 72 hours, and the medium lacks nutrients. The symbiotic action of milk kefir using soluble sprouted extracts is mutual, as the microorganisms it contains grow synergistically and create favorable changes in pH and acidity, depletion of nutrients and the creation of an appropriate environment. To improve the texture it is recommended to combine with fruits or vegetables that contain a lot of sugar.

**Keywords:** Soluble Extract, Germinated, Soya , Milk Kéfir, Microbial Population, Symbiosis, Lactic Bacteria, Acetic Bacteria, Yeasts

### INTRODUCTION

Currently, people try to consume healthy foods, but their consumption is limited to the use of animal milk as a substrate for kefir, which affects the intake of lactose intolerant patients (Palencia, 2001). Milk kefir is responsible for promoting the development of beneficial bacteria in the body (intestinal microbiota) (Trujillo, 2019), the diversity of microbes and their easy adaptation to different substrates (Rodríguez-Figueroa et al., 2017), making soluble extracts of soy sprouts of vegetables. milk kefir is an ideal alternative to include in the diet of this population (Norberto et al., 2018).

Soybeans (*Glycine max*) have a high content of lipids (18-22%), protein (38-42%) and carbohydrates and are called a legume (Instituto Nacional Autónomo de Investigaciones Agropecuarias, 1996. pp. 2-11). In the inner part of the seed is the germ which represents 2% of the grain and reserve substrate (Racines, 2011. pp. 44-45). For the germination process, carbohydrates, proteins and lipids are split to convert them into simple substances such as sugars, amino acids, and energy formed by a new plant through the phytohormones gibberellins (Pita & Pérez, 1998).

(Pita & Pérez, 1998 Prado et al., (2015) mention that the most used vegetable drink as a substrate for milk kefir is a soluble soy extract due to its content in oligosaccharides (raffinose and stachyose), BAL and yeasts suitable for the stimulation of microbial growth, amino acids and peptides (Norberto et al., 2018) According to Murugkar, (2014), a soluble extract of germinated soybeans induces hydrolysis of macromolecules, thus improving the quality of soybeans.

(Prado et al., 2015) state that kefir granules are a mass of irregular shape and gelatinous texture, similar to the white-yellow color of cauliflower, composed mainly of acetic acid bacteria, lactic acid bacteria and yeasts, in which encapsulated exopolysaccharides (kefiran) were found, source of energy and exchange of growth factors and other metabolites (Montero, 2020), therefore the

present research work proposes the following objectives for the extraction of soluble soybean sprouts (*Glycine max*) at different times (0, 24, 48, 72 and 96 hours) to create populations of lactic acid, acetic acid and yeast bacteria from the initial inoculum, certain BAL, BAA and yeasts from the soluble extract of soybean sprouts (*Glycine max*) and at different times (0, 24, 48, 72 and 96 hours) inoculated with milk kefir granules.

### RESEARCH METHODOLOGY

The present experimental study was carried out in the laboratory of bromatology, biological sciences and food processing of the faculty of livestock sciences, Polytechnic School of Chimborazo, descriptive statistics were used to measure the central tendency (mean, mode and standard deviation) and dispersion measures (standard deviation) a significance test was not performed for the method used. The importance of the method used was not proven; the symbiotic behavior of milk kefir was analyzed using soybean sprouts (*Glycine max*) as substrate; 1000 mL of soluble soy extract was used as an experimental unit, 250 mL samples were used and 4 repetitions were used. During the experimental measurements, soluble extracts of sprouted soybeans were subjected to biological and physicochemical analysis of foods: sugar, fat, protein, pH and acidity; For the determination of lactic acid bacteria, acetic acid bacteria and yeasts, microbial populations were counted in the initial inoculum of soluble extracts of soy kefirada fermented at different times (0, 24, 48, 72 and 96 h), while for the determination of commensals. Activity microorganisms were counted at different time points (0, 24, 48, 72 and 96 h) in a scatter plot to summarize.

### PREPARATION OF SOYBEAN SPROUTS

Racines (2011) used a process called glass jar germination where the beans were prewashed in 3 liters of 150 ppm water for 1 minute and then soaked for 24 hours. In a dry and cool place, germination should be carried out in glass jars placed on an inclined support, to which grains are evenly added, moistened with sprinklers 3-4 times in the dark, until reaching a length of 3 cm. Leave it exposed to direct sunlight for 2 hours.

### ELABORATION OF SOLUBLE EXTRACT OF SPROUTED SOYBEANS

The method used by Fúquene and Arenas (2018) was used, which alleges that the germinated grains were confirmed under the correct conditions, followed by manual peeling of the grain cover, in addition to blanching for 5 min at 80°C; Mixed in a 1:3 ratio (soy: water at 80°C) and filtered to remove ocar, the milk liquor was pasteurized at 80°C for 15 minutes, then aseptically packaged and stored in refrigeration.

### ELABORATION OF SOLUBLE EXTRACT GERMINATED FROM SOYBEANS INOCULATED WITH KEFIR.

Sprouted soy milk is conditioned to 25°C, make sure that the kefir granules are pre-activated with animal milk. To inoculate kefir grains, add 3% soluble sprouted soybean extract to sterilized glass containers, cover with gauze to create a microaerobic environment, and ferment at 25°C for 96 hours.


## RESULTS AND DISCUSSION

### ANALYSIS OF BROMATOLOGICAL CHARACTERISTICS

#### SOLUBLE SPROUTED SOYBEAN EXTRACT

**Table 1:** Bromatological analysis of soluble extract of sprouted soybeans.

Components	Median	Fashion	Media	D.E
Total sugars (%)	5,64	-	5,57	± 0,32



Fermentable Sugars (g/L)	3,36	-	3,37	$\pm$ 0,02
Fat (%)	1,4	-	1,40	$\pm$ 0,02
Protein %(1)	6,66	-	6,66	$\pm$ 0,23

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**Made by:** Chávez Díaz, Andrea, 2023.

- D. E: Standard deviation

(1) Conversion factor of N2=6.25

### Sugars

In the determination of the sugar content of the soluble extract of sprouted soybeans, the total and fermentable sugars present in the study were analyzed and the reported value was 5.57  $\pm$  0.32% for total carbohydrates and 3.37  $\pm$  0.02 g / L for reducing sugars. Sucrose showing a difference of 2.20% is shown in Table 1. (Oyedepi, 2018) says that the decrease in carbohydrates is related to the loss of grain nutrient stores in the germination process. (Kim W. i in., 1986). p 385) in the work: "The change of oligosaccharides in the sensory properties of soy milk during germination"; establishes that the content of fermentable sugars in sucrose is 2.9 g / l in the effect of 3 days of germination.

### GREASE

Regarding the fat content of soluble sprouted soybean extract, as shown in Table 1, the average value obtained was 1.40  $\pm$  0.02%, similar to that reported in the study (Jiang et al., 2013.p.2). : "Improvement of the food quality of soy milk made from sprouted soybeans in the short term"; Reached fat content value of 1.33%. In addition, in (Bansal, R & Kaur, M., 2015) research paper: "Improving the quality and sensory evaluation of soy milk produced from sprouted soybeans", the fat value of sprouted soy extract is 1.28% and 1.50% of soy extract. . , the difference in this value is according to (Murugkar, D., 2014, p. 6) is associated with a decrease in lipids during the catabolic activity of seeds during germination, in addition (Arenas, D., 2022.p.12) comments that it increases the activity of lipase, which converts fats into fatty acids by hydrolysis. and glycerin.

### PROTEIN

Table 1 shows that soluble extracts of sprouted soybeans contain an average of 6.66  $\pm$  0.23% protein. (Murugkar, D., 2014. p. 1) mentions in his research "Effect of soy germination on the chemical composition and quality of soy milk and tofu" that it has less protein content in values of 4.1% in sprouted soy milk using 2 days of germination. The difference in values was due to the short germination, which did not allow the correct release of proteins. The ideal time for germination is 3 to 4 days, when the level of soluble nitrogen increases (Álvarez, 2012. p. 31).

### ANALYSIS OF PHYSICOCHEMICAL CHARACTERISTICS

**Table 2:** Physicochemical analysis of soluble extract of sprouted soybeans.

Components	Median	Fashion	Media	D.E
PH	6,67	-	6,67	$\pm$ 0,04
Acidity (%)	0,18	0,18	0,17	$\pm$ 0,01

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**Made by:** Chávez Díaz, Andrea, 2023.

- D. E: Standard deviation

### PH

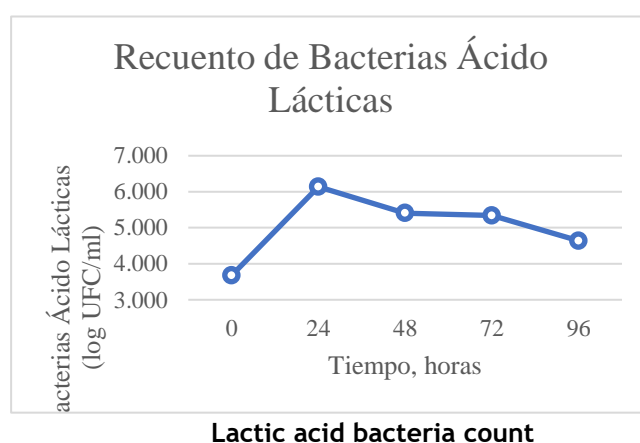
The pH value of soluble sprouted soybean extract was  $6.67 \pm 0.04$ ; a value close to (Jiang et al., 2013, p. 3) In their study "Short-term improvement of the food quality of soy milk produced from sprouted soybeans"; according to (Chavarria, M., p. 2010) obtained a value of 6.51. 15) Study "Determination of the expiration date of soy milk through real-time research"; Soluble extracts of sprouted soybeans are considered a slightly acidic product similar to animal milk.

Also (Bansal, R & Kaur, M., 2015) in his work: "Improvement of the quality and sensory evaluation of soy milk produced from germinated soybeans"; in the soluble extracts of germinated soybeans pH 6.85 was obtained and in the soluble extract without germinating pH 7.05, which decreased with germination. One of the most fundamental enzymes in soybean seed germination is phytase, which is activated at soaking and is responsible for the sequential hydrolysis of phytic acid (Rodríguez, L. and Miranda, E., 2018 p.11).

### ACIDITY

As shown in Table 2, the acidity of soluble extract of sprouted soybeans presented a lactic acid percentage of  $0.17 \pm 0.01$ . The values are similar (Valencia, 2018, p. 145), as in his study; "Technical evaluation of soft drinks and purification of traditionally fermented and pasteurized beverages based on purple corn (*Zea mays*) and quinoa (*Chenopodium quinoa*) var. INIA 420 black collana", found 0.18% (expressed in lactic acid)) purple sprouts. The low acidity of the inoculum can be explained by the high pH values obtained in this study, since there is a direct correlation between pH and acidity, which can be partially explained (Boyer, 2015 pg 1) Who said that low-acid foods usually have a pH greater than 4.6 and that vegetables, Beans and meat are foods low in acidity.

### MICROBIAL COUNT FROM THE INITIAL INOCULUM AT DIFFERENT TIMES (0, 24, 48, 72 AND 96).



**Lactic acid bacteria count**  
**Graph 1:1** Lactic Acid Bacteria Count at 0, 24, 48, 72 and 96 hours.  
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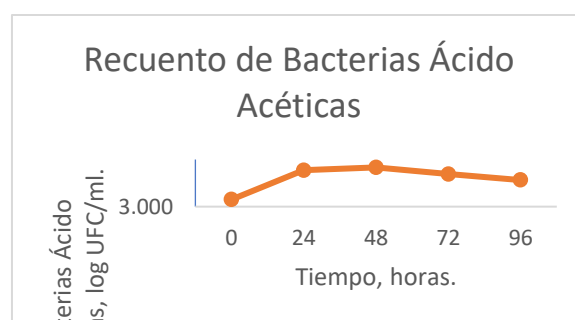
Figure 1 shows the count of lactic acid bacteria where the inoculum at hour 0 was  $3.677 \times 10^3 \pm 3.176$  log UFC/ml. The values are lower than those reported by Baú *et al.*, (2015) in the research work "Changes in soy milk during fermentation with kefir cultures: hydrolysis of oligosaccharides and production of aglycone isoflavones"; showed a figure of  $5.81 \times 10^1$  log CFU/ml, this value is related to the fact that kefir granules have demonstrated the ability to adapt to soluble soybean extracts due to earlier seeding compared to this study, it is possible that these conditions allow to improve the initial number of lactic acid bacteria, similar to the number (Norberto et al. al., 2018)

suggested an initial count of  $7.5 \times 10^3$  log CFU/ml of lactic acid bacteria, this value may be related to the use of commercial cultures directly added to the soluble soy extract and the duration of induction took less than 2.5 hours to accommodate the substrate (Baú *et al.*, 2015), further (Gamba R., *et al.* 2020) indicated that the initial population of lactic acid bacteria was influenced by the bacterial composition of the initial grains.

At 24 hours, an exponential logarithmic growth phase was observed in which  $6,139 \times 10^3 + 5,131$  log CFU/ml was obtained, contrary to the data presented in their study (Abdolmaleki *et al.*, 2015 p.444); "Evaluation of beverages made with milk, soy milk and whey using Iranian kefir starter culture", where  $8 \times 10^3$  log UFC/ml showed an increase of 15 to 24 hours when soy carbohydrates (sucrose and raffinose). They consumed lactic acid bacteria, which directly affects their population growth.

Microbial growth reached a stationary phase after 48 hours with an average of  $5.405 \times 10^3 \pm 3,833$  log CFU/ml, which is low compared to the values proposed by (Yugsi, 2022) in the study: "Evaluation of the physicochemical, microbiological and sensory properties of kefir made with almond milk (*Prunus dulcis*) and kefir made with soy milk (*Glycine max*)" indicates that the value of lactic acid bacteria will be  $7.88 \times 10^3$  log CFU/ml, which is comparable to (Abdolmaleki *et al.*, 2015)  $7.80 \times 10^1$  log CFU/ml, (Orozco, 2011). They mentioned that a stationary phase begins after 30 h of fermentation, showing that lactic acid bacteria slowly produce lactic acid and acetic acid when mixed with yeast compared to lactic acid bacteria in milk. Fermented. After 72 hours it was still on the plateau and showed a decrease in lactic acid colonies of  $5339 \times 10^3 - 4178$  log CFU/ml. (Fernández *et al.*, 2017, p. 22) reported similar values in their study: "Evaluation of the content of isoflavones and total phenols in soy milk fermented with kefir during storage and after a simulated digestion system in vitro"; which shows an approximate value of  $4 \times 10^3$  log CFU/ml and a minimum plateau at 72 hours of fermentation, which is reasonable; because according to (Suriash *et al.*, 2020 p. 136) showed that extended incubation times of 48 and 72 hours resulted in significant reductions in microbial populations as the pH of the medium decreased. However, after 96 hours there was evidence of a decrease in lactic acid bacteria in the original inoculum, as shown in Figures 1-3. in the figure, with a value of  $4.636 \times 10^3 + 3398$  log CFU/ml; Opposite numbers were obtained in his study: "Evaluation of isoflavones and total phenolic content in soy milk fermented with kefir during storage and after the in vitro simulated digestion system", where I show low numbers of  $3.9 \times 10^3$  log CFU/ml, I obtain an inverse relationship. where, in fact, the longer the resting period, the lower the bacterial content of lactic acid, competition and new adaptations between lactic acid bacteria, microorganisms that require abundant nutrients to grow, and competition for nutrients between microorganisms is a source of lactic acid stress. bacteria (Norberto, *et al.*, 2018. p. 1).

#### ACETIC ACID BACTERIA COUNT



**Figure 2.** Acetic Bacteria count at 0, 24, 48, 72 and 96 hours.

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At 0 hours,  $3,602 \times 10^3 + 2,912$  log CFU/mL was obtained, which is lower than that reported by (Fernandes, *et al.*, 2017. p.22) as it was in their study "Evaluation of the content of isoflavones and total phenols in soy milk fermented with kefir during storage and after in vitro simulation of the

digestive system". Similar values to the study conducted by (Baú, *et al.*, 2015. p.3) in "Changes in soy milk during fermentation of kefir cultures: oligosaccharide hydrolysis and aglycone isoflavone production" reported  $5.91 \times 10^1$  log CFU/ml of acetic acid bacteria due to the use of lyophilized mixed cultures in these two studies, which ensured the viability of microorganisms. This is due to the aforementioned initial populations (Gamba, R., *et al.* 2020) of lactic acid bacteria and acetic acid bacteria that are influenced by the microbial content of the initial granules.

At 24 hours, the first inoculum shows a logarithmic growth of *Acetobacter*, as shown in graphs 2-3. It reports a population average of  $6,094 \times 10^3 \pm 5,603$  log CFU/ml. This value is lower than that reported by (Baú, *et al.*, 2015. 3), who obtained a population of  $7.8 \times 10^1$  log CFU/ml for 'soymilk'. Fermented in kefir and grown from 5 hours. 2020) stated that the low sugar concentration in soy milk limits yeast growth and affects the proper growth of acetic acid bacteria that require ethanol produced by yeast.

In graph 2 it can be seen that at 48 hours the acetic acid bacteria presented the highest phase of exponential logarithmic growth in the fermentation of soy extract dissolved with kefir, with a microbial count of  $6.329 \times 10^3 \pm 5.081$  log CFU / ml, a value similar to that reported by (Fernandes, *et al.* al., 2017) where  $5.8 \times 10^3$  log CFU / ml was obtained in 48 hours of fermentation. 1) mentioning that, after 48 hours, yeast is responsible for the production of ethanol, converted into acetic acid and glycerol by acetic bacteria.

After 72 hours of fermentation, according to graph 1, a decrease in the population of acetic bacteria is entered into the stationary phase with an average of  $5.763 \times 10^3 \pm 4.788$  log CFU/ml; this may be due to a decrease in pH and increased consumption of substrate, which slows down the process of microbial activity of acetate bacteria; because the optimum pH of growth is between 5 and 6.

At 96 hours it can be seen in graph 2-3 that acetic acid bacteria are in stationary phase, due to a decrease in the population with an amount of  $5,259 \times 10^3 \pm 3,780$  log CFU / ml, the value is the same as obtained (Gamba, R., *et al.*, 2020) with count  $4.983 \times 10^1$  log CFU / ml at 96 hours in his study "Chemical characterization, Microbiological and functional kefir produced from cow's milk and soy milk. 3) The limitation of growth is produced by accumulation of acids and wear of sugars present in the medium.

#### YEAST COUNT



**Graph 32:** Yeast count at 0, 24, 48, 72 and 96 hours.

**Made by:** Chávez Díaz, Andrea, 2023.

Results of yeast counts over time are shown in soluble sprouted soybean extracts containing kefir granules. A value of  $5.209 \times 10^3 \pm 4.719$  log CFU/ml was reported at time 0. A similar value was reported by (Norberto *et al.*, 2018), who showed a microbial count of  $5.9 \times 10^3$  log CFU/ml in their study. Fermentation and growth of kefir microorganisms", high initial value (Lui, J & Lin, C., 2000: p. 716).



At 24 hours, a slight growth of the microorganism was identified as it adapted to the environment, reaching a value of  $5,576 \times 10^3 \pm 5,028$  log CFU/mL, a value similar to that reported by Gamba et al., (2020) in the study: "Chemical, microbiological and functional characterization of kefir obtained from cow's milk and soy"; with an amount of  $5,021 \times 10^1$  log cfu/ml. Validated (Je-Ruei & Chin-Wen, 2008) in the study: "Production of kefir from soy milk with or without added glucose, lactose or sucrose"; showing a growth of  $5.2 \times 10^3$  log cfu/ml. Justified (Grao, 2020), who points out that after 24 hours there is a high growth of lactic acid bacteria, the pH of the medium descends due to the formation of lactic acid, which prevents the development of yeast.

After 48 hours, the exponential phase of the yeast begins to develop and reaches a value of  $6,250 \times 10^3 \pm 5,013$  log CFU/ml, similar value was reported by (Fernandes, *et al.*, 2017) in "Evaluation of the content of isoflavones and total phenols in soy milk fermented with kefir during storage and after in vitro simulation of the digestive system" presenting a population of  $5.7 \times 10^3$  log CFU/ml. This is explained by mentioning (De la Mano, 2021), which confirms that yeasts benefit from the acidified medium produced by bacteria, using lactic acid produced by B AL as a carbon source (Grao, 2020).

Thus, after 72 hours of fermentation, the yeast entered the quiescent phase reporting a value of  $5,774 \times 10^3 \pm 4,491$  log CFU/ml, a value similar to that reported by (Fernandes, *et al.*, 2017. p.22) in their study "Evaluation of the content of total isoflavones and phenols in soy milk fermented with kefir during storage and after in vitro simulation of the digestive system" reported the amount of  $5.8 \times 10^3$  log CFU/ml. This will be stated (Laureys & Vuyst, 2016. p.1) that states that the substrate is abundant at the beginning of fermentation, but after 48 hours the concentration decreases rapidly. So after 72 hours most carbohydrates are consumed causing the microbial population to decrease.

At 96 hours a more significant decrease in yeast was observed in the initial inoculum as shown in graph 3-3 with a value of  $5,397 \times 10^3 \pm 4,674$  log CFU/ml, this value is similar to that presented by (Gamba *et al.*, 2020) in his research: "Chemical, microbiological and functional characterization of kefir made from cow's milk and soy milk", where he showed a weak growth of  $4.415 \times 10^1$  log CFU/ml. According to (Yugsi, 2022. p. 57) the extension of the long incubation period significantly reduces the amount of yeast in kefir due to a decrease in the pH of the medium because after 72 hours most carbohydrates are consumed.

#### YEAST COUNT



**Graph 33:** Yeast count at 0, 24, 48, 72 and 96 hours.

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The results of yeast counts over time in the soluble extract of soybeans sprouted with kefir granules are shown. Where at 0 hours a value of  $5209 \times 10^3 \pm 4719$  log CFU/mL was reported; Similar values are reported by (Norberto et al., 2018) who in their work "Impact of partial and total replacement of milk by soy extract in aqueous solution on the fermentation and growth parameters of kefir microorganisms", the initial high values could be due to the argument of (Lui, J & Lin, C., 2000: p. 716) where he explains that the microflora contained in the kefir grain is transferred to the soy

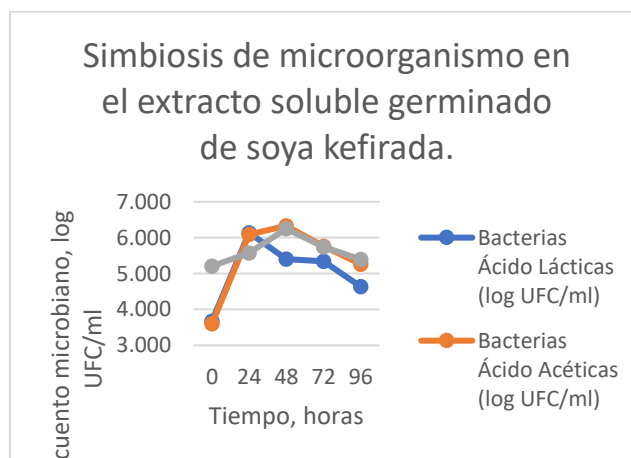
milk, depending on the active microorganism before planting, the one after inoculation in soy milk grows slowly compared to animal milk.

At 24 hours, a slight growth of the microorganism was noted as it adapts to the environment, reaching a value of  $5,576 \times 10^3 \pm 5,028$  log CFU/ml, a value similar to that reported by Gamba et al., (2020) in the study: "Chemical, microbiological and functional characterization of cow's milk kefir and soy milk"; with a count of  $5,021 \times 10^1$  log CFU/ml. Confirmed by (Je-Ruei & Chin-Wen, 2008) in the study: "Production of kefir from soy milk with or without added glucose, lactose or sucrose"; representing a growth of  $5.2 \times 10^3$  log CFU/ml. Justified by (Grao, 2020) who mentions that after 24 hours there is evidence of a strong growth of lactic acid bacteria, the pH of the medium decreases due to the production of lactic acid, which hinders the development of yeasts.

After 48 hours, the exponential phase of the yeast began to develop and reached values of  $6250 \times 10^3 \pm 5013$  log CFU/ml, (Fernandes, et al., 2017) reported similar values in the section "Evaluation of Isoflavone Content". "and total phenols in stock in soy milk fermented with kefir during and after in vitro simulation of the digestive system", with a population of  $5.7 \times 10^3$  log CFU/ml. This is in agreement with the aforementioned explanation (De la Mano, 2021), which confirmed that yeast benefits from an acidified medium produced by bacteria that use the lactic acid produced by BAL as a carbon source (Grao, 2020). Thus, after 72 hours of fermentation, the yeast entered the stationary phase and reported a value of  $5774 \times 10^3 \pm 4491$  log CFU/ml, corresponding to (Fernandes, et al., 2017 p.). 22) In their study "Evaluation of the content of isoflavones and total phenols in soy milk fermented with kefir during storage and after in vitro simulation of the digestive system" a figure of  $5.8 \times 10^3$  log CFU/ml was reported. This will be determined (Laureys & Vuyst, 2016 Page 1) Who mentions that at the beginning of fermentation the substrate is abundant, but after 48 hours the concentration drops rapidly. In this way, most carbohydrates are consumed after 72 hours, which translates into a decrease in the microbial population.

After 96 hours, the initial inoculum showed a significant reduction in yeast as shown in Figure 3-3. in the figure, with values of  $5397 \times 10^3 \pm 4674$  log CFU/ml, and these values correspond to (Gamba et al. ..., 2020) in their study: "Chemical, microbiological and functional properties of kefir prepared from cow and soy milk" showed a slight increase of  $4,415 \times 10^1$  log CFU/ml. According to (Yugsi, 2022. Page 57) Long incubation times significantly reduced the yeast population in kefir due to a drop in the pH of the medium, as most carbohydrates were consumed after 72 hours.

#### SYMBIOTIC BEHAVIOR OF SOLUBLE SOY EXTRACT SPROUTED WITH MILK KEFIR



**Gráfico 1:** Comportamiento simbiótico del Extracto soluble de soja germinado inoculada con granos de kéfir en diferentes tiempos.

**Realizado por:** Chávez Díaz, Andrea, 2023.



Results obtained after 0 hours of symbiosis showed the number of different microbial populations with reported numbers of  $3677 \times 10^3$  3176 log CFU/ml for lactic acid bacteria,  $3602 \times 10^3$  2912 log CFU/ml for acetic acid bacteria and  $3602 \times 10^3$  5 9X/ml. log CFU/ml; The populations of lactic acid bacteria and acetic acid bacteria were similar, while the yeast growth rate was higher (Figure 4-3), (Lui, J & Lin, C, .2000: p. 716) At this point he showed that he explains that microbes transfer acids from kefir grains to soy milk based on the microorganisms that were active before planting. In addition, the soluble extract of germinated soybeans initially inoculated with kefir presented a pH lower than 6.67, which was optimal for its development. , and according to (Contreras, R., 2014. page 2) Yeast grows in an environment with a slightly acidic pH level. Likewise, these values differ from those reported (Baú, et al., 2015, p.3) in the study "Changes in soy milk during fermentation with kefir culture: hydrolysis of oligosaccharides and production of aglycone isoflavones". , where 0 hours I reported  $7.8 \times 10^1$  log CFU/ml for lactic acid bacteria,  $7.4 \times 10^1$  log CFU/ml for acetic acid bacteria and  $6 \times 10^3$  log CFU/ml for yeast because she also used freeze-dried mixed cultures in her study.

As seen in Figure 4, lactic acid bacteria and acetic acid bacteria showed high growth after 24 hours with numbers of  $6139 \times 10^3$  5131 and  $6094 \times 10^3$  5603 log CFU/ml, respectively; while the yeast grew slowly with a value of  $5 \times 10^2$ , 57 log CFU/ml. It is worth mentioning that during this time, lactic acid bacteria reached the highest point of growth (exponential phase) compared to other strains, which was related to the rapid consumption of soy carbohydrates (sucrose and raffinose) by lactic acid bacteria, which directly affects their population. increased and therefore has a high growth rate as described in (Abdolmaleki et al., 2015). 444). These numbers agree with (Fernades et al., 2017. page 22) "Evaluation of isoflavones and total phenolic content in soy milk fermented with kefir and simulation of the digestive system" I reported  $8.3 \times 10^3$  log CFU/ml at 24 hours for lactic acid bacteria and  $7.8 \times 10^3$  log CFU/ml for acetic acid bacteria,  $5.7 \times 10^3$  log CFU/ml in yeast, Reduced pH 4.54. A 48-hour count in this study showed an initial decrease in lactic acid bacteria and an exponential increase in acetic acid bacteria and yeast with numbers of  $5405 \times 10^3$  3833 log CFU/ml; 6329 5081 log CFU/ml and  $6250 \times 10^3$  log CFU/0103 ml. These data differ from those obtained (Fernades et al., 2017, p.23) "Evaluation of the content of isoflavones and total phenols of soy milk fermented with kefir and after simulation of the digestive system". Among them, after 48 hours of fermentation, the value of lactic acid bacteria was  $8.5 \times 10^3$  log CFU/ml, the value of acetic acid bacteria was  $5.8 \times 10^3$  log CFU/ml and the value of yeast was  $5.7 \times 10^3$  log CFU/ml. Explained by (Grao, 2020), who commented that during fermentation, lactic acid bacteria will multiply rapidly, produce lactic acid, lower pH, high lactic acid production can inhibit bacterial growth, yeast uses lactic acid as a carbon source, reducing its concentration and increasing the pH value, which creates a suitable environment for the continuous growth of lactic acid bacteria and acetic acid bacteria.

After 72 hours, both acetic bacteria and yeasts entered the stationary phase and reported values of  $5763 \times 10^3 \pm 4788$  and  $5744 \times 10^3 \pm 4491$  log CFU/ml, respectively; while the number of lactic acid bacteria continued to decrease after 48 hours. from  $5339 \times 10^3$  4178 log CFU/ml. This reduction in the number of microorganisms has been demonstrated (Suriash et al., 2020). p.136) who stated that prolonged incubation periods of 48 and 72 hours significantly reduced the number of microorganisms due to the decrease in the pH of the medium in which they were placed. This was confirmed by (Laureys & Vuyst, 2014), who indicated that the main metabolites produced during fermentation were ethanol and lactic acid, resulting in low concentrations of glycerol, acetic acid and mannitol produced after 72 h of fermentation. The pH drops to 3.45, which is a more limiting factor that reduces the survival and development of bacteria and yeast. After 96 hours of fermentation, the microbial count showed a more pronounced decrease, reporting values of  $4.636 \times 10^3 \pm 3.398$  log CFU/ml in lactic acid bacteria, while in acetic acid bacteria and yeasts showed similar values of  $5.259 \times 10^3 \pm 3.103$  and  $5397 \times 10^3 \pm 4, 674$  log CFU/ml. According to (Miranda, D., 2018, p. 32) The lower reduction of LAB may be due to microbial interactions that normally occur between microorganisms, resulting in competition, symbiosis or symbiosis; LAB inhibition is often

caused by yeasts, as these microorganisms are usually present in a sequential order (yeasts precede LAB). Yes, once the sugars have been fermented and almost all available nutrients have been consumed, the yeast and bacteria begin their cell death. phase after 96 hours, facilitating the dominance of new microbes in the environmental state.

**Table 3.** Symbiotic behavior of soluble extract of sprouted soybeans inoculated with kefir grains at different times (0, 24, 48, 72 and 96 hours)

		Microbial Growth Time (Hours)				
		0	24	48	72	96
<b>Lactic Acid Bacteria</b> (log CFU/ml)	Median	3,699X10 <sup>3</sup>	6,139X10 <sup>3</sup>	5,406X10 <sup>3</sup>	5,334X10 <sup>3</sup>	4,638X10 <sup>3</sup>
	Fashion	3,778X10 <sup>3</sup>	-	-	-	-
	Q. E	± 3,176	± 5,131	± 3,833	± 4,178	± 3,398
	Media	3,677X10 <sup>3</sup>	6,139X10 <sup>3</sup>	5,405X10 <sup>3</sup>	5,339X10 <sup>3</sup>	4,636X10 <sup>3</sup>
<b>Acetic acid bacteria</b> (log CFU/ml)	Median	3,602X10 <sup>3</sup>	6,022X10 <sup>3</sup>	6,325X10 <sup>3</sup>	5,784X10 <sup>3</sup>	5,259X10 <sup>3</sup>
	Fashion	3,602X10 <sup>3</sup>	-	-	-	-
	Q. E	± 2,912	± 5,603	± 5,081	± 4,788	± 3,780
	Media	3,602X10 <sup>3</sup>	6,094X10 <sup>3</sup>	6,329X10 <sup>3</sup>	5,763X10 <sup>3</sup>	5,259X10 <sup>3</sup>
<b>Levaduras (log</b> <b>UFC/ml)</b>	Median	5,227X10 <sup>3</sup>	5,622 X10 <sup>3</sup>	6,250X10 <sup>3</sup>	5,744X10 <sup>3</sup>	5,410X10 <sup>3</sup>
	Fashion	-	-	-	-	-
	Q. E	± 4,719	± 5,028	± 5,013	± 4,491	± 4,674
	Media	5,209X10 <sup>3</sup>	5,576X10 <sup>3</sup>	6,250X10 <sup>3</sup>	5,744X10 <sup>3</sup>	5,397X10 <sup>3</sup>

Made by: Chávez Díaz, Andrea, 2023.

The values obtained in the bromatological analysis using the soluble extract of germinated soybeans showed a total carbohydrate content of 5.57%, but only 3.37 g / L correspond to fermentable sugars usable by the microorganisms source of kefir as food and energy, as well as lipids (1.40%) and proteins (6.66%). As for the physicochemical properties, its pH (6.67) is ideal for use in fermented beverages, being a non-dairy drink, as well as its low acidity (0.17% lactic acid).

- The initial inoculum count of soluble soybean extract sprouted with kefir granules reached its exponential logarithmic development phase at 24 hours in lactic acid bacteria (6,139 x 10<sup>3</sup> log CFU/ml), while in acetic acid bacteria (6,329 x 10<sup>3</sup> log CFU/ml) and yeast (6,250 x 10<sup>3</sup> log CFU/ml) after 48 hours; Microbial population decline begins after 72 hours for lactic acid bacteria (5339 x 10<sup>3</sup> log CFU/ml), acetic acid bacteria (5763 x 10<sup>3</sup> log CFU/ml) and yeast (5744 x 10<sup>3</sup> log CFU/ml).
- As for symbiotic activity, lactic acid bacteria reach their maximum growth after 24 hours, lowering the pH of the medium, while yeast growth is stimulated after 48 hours, favoring the growth of acetic acid bacteria and creating an environment conducive to the maintenance of lactic acid bacteria. The acid is suitable for bacteria that produce a dormant state for 48 to 72 hours. Once the sugars have fermented and virtually all available nutrients have been consumed, after 96 hours the bacteria and yeasts represent a significant decrease in the population as they begin their stage of cell death, creating a relationship of reciprocity from which bacteria and yeasts benefit from the growth to their eventual disappearance.

## RECOMMENDATIONS

- Preinoculate kefir grains with sprouted soybean extract to acclimatize the microorganisms to the substrate. • Allow soybeans to germinate for no more than 5 days to take advantage of germination. • To increase the microbial population it is necessary to add vegetable substrates with a higher sugar content, for example: fruits or vegetables with a high fermentable sugar content, so it is necessary to choose to add some sweeteners that favor the growth of microbes. .
- There is little information on soluble extracts of sprouted legumes, so further research is recommended on the use of nutrients in sprouted legumes such as soybeans, beans, chickpeas, beans, etc. for the preparation of probiotic drinks. • Due to the lack of information on sprouted soy drinks, physicochemical, food and microbiological analyses of the dry extract are recommended

## BIBLIOGRAPHY

- [1] **ABDOLMALEKI, Farzaneh, et al.** "Assessment of beverages made from milk, soya milk and whey using Iranian kefir starter culture". International Journal of Dairy Technology [en línea]. 2015. (Teherán-Irán) 68 (3), pp. 441-447. Disponible en: <https://onlinelibrary.wiley.com/doi/abs/10.1111/1471-0307.12197>
- [2] **ALVAREZ, Yenny.** Development and characterization of two protein drinks, one based on malted quinoa and the other based on unmalted quinoa (*Chenopodium quinoa*) [Online]. (Degree Work). (Food Industry Engineer) Universidad Nacional Jorge Basadre Grohmann-Tacna, Facultad de Ciencias Agropecuarias, Escuela Académico Profesional de Ingeniería en Industrias Alimentarias, Perú. 2012. [Accessed: 1 October 2022]. Available in: [http://repositorio.unjbg.edu.pe/bitstream/handle/UNJBG/2920/17\\_2012\\_alvarez\\_carita\\_yc\\_fcag\\_industrias\\_alimentarias.pdf?sequence=1&isAllowed=y](http://repositorio.unjbg.edu.pe/bitstream/handle/UNJBG/2920/17_2012_alvarez_carita_yc_fcag_industrias_alimentarias.pdf?sequence=1&isAllowed=y)
- [3] **ARENAS VELA, Dilfer.** Influence of germination on the chemical-proximal composition, bioactivity, minerals and thermal properties of lentils (*Lens culinaris M.* ), peas (*Pisum sativum L.* ) and stick bean (*Cajanus cajan L.* ) [Online] (Degree work) (Engineering). Universidad Agraria de la Selva, Faculty of Engineering in Food Industries, Professional School of Engineering in Food Industries. Tingo Maria-Peru. 2022. pp. 12-33-34. [Accessed: 2023-01-06]. Available in: [http://repositorio.unas.edu.pe/bitstream/handle/UNAS/2246/TS\\_DAV\\_2022.pdf?sequence=1&isAllowed=y](http://repositorio.unas.edu.pe/bitstream/handle/UNAS/2246/TS_DAV_2022.pdf?sequence=1&isAllowed=y)
- [4] **BANSAL, R. & KAUR, M.** "Quality improvement and sensory evaluation of soya milk prepared by germinated soybeans". Semantic Scholar [En línea]. 2015. Disponible en: <https://www.semanticscholar.org/paper/QUALITY-IMPROVEMENT-AND-SENSORY-EVALUATION-OF-SOYA-Bansal-Kaur/f571e8f67338e7eec28f6a6f1623953bbfea924d>
- [5] **BAÚ, T.R; et al.** "Changes in soymilk during fermentation with kefir culture: oligosaccharides hydrolysis and isoflavone aglycone production". International Journal of Food Sciences and Nutrition [en línea]. 2015. 66(8). ISSN: 1465-3478. Disponible en: <https://pubmed.ncbi.nlm.nih.gov/26460145/>
- [6] **BOYER, Renee.** Elaboration of Pressure Preserves. Elaboration of Pressure Preserves Rev. Virgine cooperative extension. [Online] 2015. Virginia. p. 1. Available in: <https://vtechworks.lib.vt.edu/bitstream/handle/10919/75372/348-5855.pdf?se>
- [7] **CASTRO, Jennifer & CHÍA, Liseth.** Evaluation of the nutritional contribution and health risks associated with the consumption of sprouts. [Online] (Degree work) (Engineering). University of Pamplona, Faculty of Engineering and Architecture, Specialization in food safety Bucaramanga. Pamplona- Spain. 2020. p. 46. [Accessed: 21 August 2022.]. Available in: <http://repositoriodspace.unipamplona.edu.co/jspui/bitstream/20.500.12744/4301/1/Castro%20Chia%2020%20TG.pdf>
- [8] **CHAMPI, Faustino and TAYPE, Nataly.** 2018. Effect of cooking and fermentation time on the quality of fermented beverage based on purple corn (*Zea Mays*) germinated from Kculli variety. (Degree work). (Food Industry Engineer). National University of San Agustín de Arequipa, Faculty of Process Engineering, Professional School of Food Industry Engineering. Arequipa, Peru. [Online] 2018. [Accessed: October 21, 2022]. Available in:

- <http://repositorio.unsa.edu.pe/bitstream/handle/UNSA/7227/IAchchf.pdf?sequence=1&isAllowed=y>.
- [9] **CHAVARRÍ MORBIONI, María Lorena.** Determination of the shelf life of soy milk through a real-time study. [Online] (Degree work) (Technologist). Polytechnic School of the Littoral. Food Technology Specialization Program. Guayaquil - Ecuador. 2010. p. 8. [Accessed: 2023-01-17]. Available in: [https://www.dspace.espol.edu.ec/bitstream/123456789/9057/1/TESIS%20LECHE%20DE%20SOYA%20LORENA%20CHAVARRIA.pdf?fbclid=IwAROVqTIUL9dN3XAPWXVleU2nriyYa-kcyNbWPMNrX1OoaleYUfny-p3\\_qc0](https://www.dspace.espol.edu.ec/bitstream/123456789/9057/1/TESIS%20LECHE%20DE%20SOYA%20LORENA%20CHAVARRIA.pdf?fbclid=IwAROVqTIUL9dN3XAPWXVleU2nriyYa-kcyNbWPMNrX1OoaleYUfny-p3_qc0)
- [10] **CCOYLLO, Noe.** Development of a carbonated energy drink from quinoa (*Chenopodium quinoa*), Kañihua (*Chenopodium pallidicaule*) and barley (*Hordeum vulgare*) malts. (Degree Work) (Food Industry Engineer). National University of San Cristóbal de Huamanga, Faculty of Chemical Engineering and Metallurgy, Professional School of Engineering in Food Industries, Peru. 2019. [Accessed: 8 November 2022]. Available in: <http://repositorio.unsch.edu.pe/handle/UNSCH/3409>
- [11] **CONTRERAS, Ramon.** Means for growing yeasts [Online] Biology. 2014. p. 2. [Accessed: 11 January 2023]. Available in: <https://biologia.laguia2000.com/hongos/medios-para-crecer-levaduras>
- [12] **HAND IN HAND, Luciana Gabriela, et al.** "Description and analysis of kefir production techniques". Revista Nutrición Investiga [Online]. 2021. (Buenos Aires). Available in: [http://escuelanutricion.fmed.uba.ar/revistani/pdf/21b/an/927\\_c.pdf](http://escuelanutricion.fmed.uba.ar/revistani/pdf/21b/an/927_c.pdf)
- [13] **ESQUIVEL RIVERA, Ingrid Ninibeth & CAYRO ORTEGA, Stephany Samantha.** "Elaboration of gluten-free bread based on rice flours (*Oryza sativa*), soy (*Glycine max*) and malted lentils (*Lens culinaris*), design and evaluation of an industrial fermenter". [Online] (Degree Work) (Engineering) (Undergraduate Thesis) Catholic University of Santa Maria. Faculty of Biological and Chemical Sciences and Engineering. Professional School of Food Industry Engineering. Peru. 2018. p. 17. [Accessed: 2023-01-08]. Available in: <https://core.ac.uk/download/pdf/198131445.pdf>
- [14] **FERNANDES, Meg, et al.** Evaluation of the content of isoflavones and total phenols of soy milk fermented with kefir during storage and after in vitro simulation of the digestive system. Food Chemistry[en línea]. 2017. Disponible en: doi: <http://dx.doi.org/10.1016/j.foodchem.2017.02.095>
- [15] **FUQUENE, Jessica & ARENAS, Nathalya.** Development of a fermented and flavored soy-based beverage with the addition of inulin and probiotic cultures [online]. (Degree Work). (Food Engineering) De La Salle University, Faculty of Engineering, Unisalle Science, 2018. Bogota. Available in: [https://ciencia.lasalle.edu.co/cgi/viewcontent.cgi?article=1178&context=ing\\_alimentos](https://ciencia.lasalle.edu.co/cgi/viewcontent.cgi?article=1178&context=ing_alimentos).
- [16] **GAMBA, Raúl, et al.** "Chemical, Microbiological, and Functional Characterization of Kefir Produced from Cows Milk and Soy Milk". International Journal of Microbiology[en línea]. 2020. 2020. Disponible en: <https://www.hindawi.com/journals/ijmicro/2020/7019286/>
- [17] **GERALD, Liliana Mabel.** Characterization of acetic acid bacteria intended for the production of fruit vinegars. [Online] (Degree work) (Doctorate). Polytechnic University of Valencia. Department of Food Technology. Spain. 2015. p. 33. [Accessed: 2023-01-10]. Available in: <https://riunet.upv.es/bitstream/handle/10251/59401/GERARD%20-%20Caracterizaci%C3%B3n%20de%20bacterias%20del%20C3%A1cido%20ac%C3%A9tico%20destinadas%20a%20la%20producci%C3%B3n%20de%20vinagres%20de....pdf?sequence=1>
- [18] **GRAO, Christian.** Microbiology, composition and benefits of kefir[online]. (Degree work). Complutense University, Faculty of Pharmacy. 2020. Available in: <http://147.96.70.122/Web/TFG/TFG/Memoria/CRISTINA%20GRAO%20ROLDAN.pdf>.
- [19] **NATIONAL AUTONOMOUS INSTITUTE OF AGRICULTURAL RESEARCH.** Soybean Cultivation Manual [online]. Ecuador, Boliche experimental station, 1996. Available in: <https://repositorio.iniap.gob.ec/bitstream/41000/2076/1/iniaplsm32.pdf>
- [20] **JE-RUEI, Liu & CHIN-WEN, Lin.** "Production of Kefir from Soymilk with or without added glucose, lactos, or sucrose". Journal of Food Science[en línea]. 2008. 65 (4). pp. 716-719. Disponible en: <https://ift.onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2621.2000.tb16078.x>
- [21] **JIANG, Susu, CAI, Weixi y Xu, BAOJUN.** "Food Quality Improvement of soy milk made from short-time germinated soybeans". PubMed Central[en línea]. 2013. (China) 2(2), pp. 198-212. Disponible en:



- <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5302266/#:~:text=Short-time%20germination%20is%20also,of%20the%20soy%20milk%20produced.>
- [22] **KIM, Woo-Jung et al.** "Changes in Oligosaccharides and Sensory Quality of Soymilk During Germination". Korean J. Food SCL, Technol [en línea]. 1986. (Korea) 18(5), pp. 384-385. Disponible en: <https://koreascience.kr/article/JAKO198603041915759.pdf>
- [23] **LAUREYS, David & De VUYST, Luc.** *Microbial Species Diversity, Community Dynamics, and Metabolite Kinetics of Water Kefir Fermentation*. Applied and Environmental Microbiology [En línea]. 2014.
- [24] **LENGKEY, H.A.W & BALIA, R.L.** *The effect of starter dosage and fermentation time on pH ad Lactic Acid Production*. Biotechnology in Animal Husbandry [En línea]. 2014, Belgrade-Zemun 30(2), pp. 339-347. ISSN 1450-9156.
- [25] **LEMON GARDUZA, Rocío Ivonne.** Obtaining foods and ingredients with antihypertensive properties from germinated and fermented legumes. [Online] (Degree Project) (Doctorate). (Postgraduate Thesis) Autonomous University of Madrid, Faculty of Sciences, Department of Agricultural Chemistry and Bromatology. Madrid. 2015. p.35. [Accessed: 2023-01-04]. Available in: [file:///C:/Users/Hello/Downloads/limon\\_garduza\\_rocio\\_ivonne.pdf](file:///C:/Users/Hello/Downloads/limon_garduza_rocio_ivonne.pdf).
- [26] **MIRANDA, Dalia.** Selection of yeasts and lactic acid bacteria native to Querétaro based on their oenological potential, compatibility and contribution to the aromatic profile of wines. [Online] (Degree work). (Engineering). Autonomous University of Querétaro, Faculty of Chemistry, Graduate Program in Food of the Center of the Republic (Propac). Santiago de Querétaro- Mexico. 2018.p. 125. Available in: <http://ri-ng.uaq.mx/bitstream/123456789/1199/1/RI007880.pdf>
- [27] **MONTERO, Luis.** Characteristics of a fermented drink made with kefir [online]. (Degree Work). (Livestock Industry Engineer) Escuela Superior Politécnica de Chimborazo, Facultad de Ciencias Pecuarias, Carrera de Ingeniería en Industrias Pecuarias, Riobamba, Ecuador. 2021. [Accessed: on: June 29, 2022]. Available in: <http://dspace.esPOCH.edu.ec/bitstream/123456789/15552/1/27T00505.pdf>.
- [28] **MURUGKAR, Dipika.** "Effect of sprouting of soybean on the chemical composition and quality of soymilk and tofu". Journal of Food Science and Technology [en línea]. 2011. (India) 51(5), pp. 915-921. Disponible en: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4008751/>
- [29] **NORBERTO, Ana et al.** "Impact of partial and total replacement of milk by water-soluble soybean extract on fermentation and growth parameters of kefir microorganisms"[en línea]. 2018, 93, pp. 491-498. Disponible en: <https://www.sciencedirect.com/science/article/pii/S0023643818302949#:~:text=Thus%2C%20these%20findings%20support%20the,vegetarians%2C%20and%20lactose%20intolerant%20people.>
- [30] **OJHA, Pravin et al.** "Effect of Sprouting in Physico-chemical Properties of Tofu". MedCrave Journal of Nutritional Health & Food Engineering [En línea]. 2014. (Nepal) 1(2). p. 3. Disponible en: [https://www.academia.edu/35646716/Effect\\_of\\_Sprouting\\_in\\_Physico\\_chemical\\_Properties\\_of\\_Tofu](https://www.academia.edu/35646716/Effect_of_Sprouting_in_Physico_chemical_Properties_of_Tofu)
- [31] **OROZCO, Fatima.** Production of lactic acid by anaerobic fermentation and its polymerization from ring opening reactions [Online]. (Degree work). (Postgraduate thesis). Centro de investigación Científica de Yucatán A.C, Mérida-Yucatán. 2011. [Accessed: 29 September 2022.] Available in: [https://cicy.repositorioinstitucional.mx/jspui/bitstream/1003/1333/1/PMP\\_M\\_Tesis\\_2011\\_Fatima\\_Orozco\\_Olivarez.pdf](https://cicy.repositorioinstitucional.mx/jspui/bitstream/1003/1333/1/PMP_M_Tesis_2011_Fatima_Orozco_Olivarez.pdf).
- [32] **OYEDEJI, Alibola, MELLEN, John & IJABADENIYI, Oluwatonsin.** *Improvement of some quality attributes of soymilk through optimization of selected soybean sprouting parameters using response surface methodology*. CyTA-Journal of Food [En línea], 2018, 16(1). ISSN:1947-6345. Disponible en: <https://www.tandfonline.com/doi/full/10.1080/19476337.2017.1388292>
- [33] **PALENCIA, Yannett.** "Dairy foods and their limitations". Naturopathic Medicine [online]. 2001. (Venezuela) (3), ISSN 1576-3080.
- [34] **PITA, Jose & PEREZ, Felix.** *Seed germination*[Online]. Madrid: Ministerio de Agricultura pesca y alimentación, 1998. Available in: [https://www.mapa.gob.es/ministerio/pags/biblioteca/hojas/hd\\_1998\\_2090.pdf](https://www.mapa.gob.es/ministerio/pags/biblioteca/hojas/hd_1998_2090.pdf)

- [35] PRADO, Maria, y otros. "Milk kefir: composition, microbial cultures, biological activities, and related products". Review article [en línea]. 2015, (Brazil), 6. Disponible en: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4626640/>
- [36] RACINES, Adriana. Research of lentil, quinoa, carrot, mustard sprouts and their application to current gastronomy [Online]. (Degree work) ( Gastronomic Manager) Equinocial Technological University, Faculty of Tourism and Environmental Preservation, Hospitality and Gastronomy, Quito-Ecuador. 2011. pp. 1-112. [Accessed: 2022-05-20]. Available in: [http://repositorio.ute.edu.ec/bitstream/123456789/11638/1/45590\\_1.pdf](http://repositorio.ute.edu.ec/bitstream/123456789/11638/1/45590_1.pdf).
- [37] RODRÍGUEZ BLANCO, Lili & MIRANDA CRUZ, Edith. Content of Phytic Acid and Inorganic Phosphorus in *Vigna unguiculata* and *Phaseolus vulgaris* Germinated at Different Temperatures. [Online] Rev.: Scielo. La Serena - Chile. 2018. p.11. [Accessed: 09 January 2023]. Available in: [https://www.scielo.cl/scielo.php?pid=S0718-07642018000300039&script=sci\\_arttext&fbclid=IwAR2J3e36QG4U0TmRuN342loiclj2P6c3fK9lAKepPtZK5INdifuNL6KQ0yk](https://www.scielo.cl/scielo.php?pid=S0718-07642018000300039&script=sci_arttext&fbclid=IwAR2J3e36QG4U0TmRuN342loiclj2P6c3fK9lAKepPtZK5INdifuNL6KQ0yk)
- [38] RODRÍGUEZ-FIGUEROA, et al. "Advances in the study of the multifunctional bioactivity of kefir". Interciencia [online]. 2017. (Venezuela) 42, pp. 347-349. ISSN 0378-1844. Available in: <https://www.redalyc.org/pdf/339/33951621003.pdf>
- [39] SURIASIH, Ketut, et al. Chemical Characteristics And Microbiological Kefir Beverages From Bali Cattle During Storage. International Journal of Scientific & Technology Research[en línea]. 2020. 9. ISSN 2277-8616.
- [40] TRUJILLO, Erika. Design of an industrial process for the production of kefir in the microenterprise of Lácteos Camilita [online]. (Degree work). (Engineering). Chimborazo Polytechnic School, Faculty of Sciences, Chemical Engineering, Riobamba-Ecuador, 2019. [Accessed: May 17, 2022.] Available in: <http://dspace.esPOCH.edu.ec/bitstream/123456789/11062/1/96T00538.pdf>.
- [41] VALENCIA, Patricia. Technological evaluation of the germination and clarification of traditional fermented and pasteurized beverages of purple corn (*Zea mays*) and quinoa (*Chenopodium quinoa*) variety Inia 420 Negra Collana. [Online] (Degree work). (Engineering). National University of San Agustín de Arequipa, Faculty of Process Engineering, Professional School of Food Industry Engineering. Arequipa - Peru. 2018.p. 125. Available in: [http://repositorio.lamolina.edu.pe/bitstream/handle/20.500.12996/1903/Q02\\_H832%20-T.pdf?sequence=1&isAllowed=y](http://repositorio.lamolina.edu.pe/bitstream/handle/20.500.12996/1903/Q02_H832%20-T.pdf?sequence=1&isAllowed=y).
- [42] YUGSI, Carla. Evaluation of the physicochemical, microbiological and sensory properties of kefir made from almond milk (*Prunus dulcis*) and kefir made from soy milk (*Glycine max*) [Online]. (Degree work). ( Biotechnology Engineering) University of the Armed Forces, Sangolquí-Ecuador.2022. [Accessed: 2 October 2022.]. Available in: <http://repositorio.espe.edu.ec/jspui/bitstream/21000/29625/1/T-ESPE-052345.pdf>.