PHYSICO-CHEMICAL, CULTURE VIABILITY, AND SENSORY FEATURES OF KEFIR ICE CREAM AS AFFECTED BY VARIOUS *Saccharomyces cerevisiae* ATCC 36858 CONCENTRATIONS

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FISIKO-KIMIA, VIABILITAS KULTUR, DAN FITUR SENSORIK ES KRIM KEFIR YANG DIPENGARUHI OLEH BERBAGAI KONSENTRASI Saccharomyces cerevisiae ATCC 36858

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ABSTRACT

The purpose of this research was to determine the character of kefir ice cream enrichment with Saccharomyces cerevisiae ATCC 36858 in various concentration. The study was conducted using a complete randomized design with 4 treatments (T0=0%; T1=0.33%; T2=0.66%; T3=1% S. cerevisiae ATCC 36858 (w/v)) and 4 repetitions. The results of the study were processed using ANOVA and DMRT. The treatment increases acidity, ethanol, viscosity and decreases the pH value, overrun, and melting rate of the product ($p<0.05$). The micro nutrients (C, O, K, Ca, P, Cl, S, Na, Mg, Si) and macro nutrients (protein and fat) has changed ($p>0.05$). The lactic acid bacteria and yeast content has decrease of 10^5 CFU/g during 28 days of storage. T2 has a good overall acceptance value with flavor and texture favored by panelists.

ABSTRAK

Tujuan penelitian ini adalah untuk mengetahui karakter es krim kefir yang diperkaya dengan Saccharomyces cerevisiae ATCC 36858 dalam berbagai konsentrasi. Penelitian dilakukan menggunakan rancangan acak lengkap dengan 4 perlakuan (T0=0%; T1=0,33%; T2=0,66%; T3=1% S. cerevisiae ATCC 36858 (w / v)) dan 4 pengulangan. Hasil penelitian diolah menggunakan ANOVA dan DMRT. Perlakuan mampu meningkatkan keasaman, etanol, viskositas dan menurunkan nilai pH, overrun, dan laju leleh produk ($p<0,05$). Kandungan mikro nutrient (C, O, K, Ca, P, Cl, S, Na, Mg, Si) dan makro nutrient (protein dan lemak) mengalami perubahan ($p>0,05$). Kandungan bakteri asam laktat dan ragi mengalami penurunan sebesar 10^5 CFU/g selama 28 hari penyimpanan. T2 adalah produk yang memiliki nilai penerimaan keseluruhan terbaik. Sebagai kesimpulan, T2 mampu menghasilkan produk dengan rasa dan tekstur yang disukai oleh panelis.

INTRODUCTION

Recently, efforts have been made to decrease the use of chemical drugs and increase lifestyle changes and natural therapies, such as by drinking kefir. Kefir that contains high probiotic is considered to be a new approach to effective treatment of various metabolic disorders. World Health Organization (WHO) defines probiotics as living microorganisms with beneficial health effects on the human body when consumed in sufficient quantities. Lactic acid bacteria (LAB) and *Saccharomyces* sp are part of the kefir grain ecosystem. Furthermore, they are able to survive over long storage periods in frozen systems such as kefir ice cream matrix that includes nutritive constituents such as milk proteins and fat.

Ice cream is a complex multiphase system that consists of dispersed air cells, coalescence fat globules, crystal ice, and aqueous phase with dissolved mineral and suspended proteins (*Indonesian National Standardization Agency,1995*). Ice cream has good potential to be used as probiotic vehicle because of its composition as well as its acceptable flavor and texture (*Agustin et al., 2021*). Recently, new ice cream formulations have been developed with various probiotics, one of them is kefir ice cream. Incorporating kefir grain into the ice cream mix (ICM) is expected to help reduce the sugar and fat contents of the probiotic ice cream. ICM made from fermented milk has a simpler sugar and fat content that significantly affects the sensory quality of probiotic ice cream (*Jardines et al., 2020*). However, there were still many shortcomings in the product including flavor, texture, melting, color, and nutritional content.

Senaka et al (2013) reported that kefir grain produces ethanol during its fermentation process, thus affecting the sensory quality of the final product. *Akalın et al* (2018) argued that enrichment on ice cream with probiotic has significantly increased the acidity of product. In addition, *Khademi et al (2022)* also reported that probiotic inside the formulation significantly affects the physicochemical and sensory properties especially fatty acid profile of sourness. On the contrary, utilization of *Saccharomyces boulardii* in combination with *Lactobacillus rhamnosus* GG in probiotic ice cream formulations gives insignificant affect to its sensory quality (*Goktas et al., 2022*). This was due to the role of *Saccharomyces* sp.

In general, *S. cerevisiae* serves to produce functional food due to their antimicrobial activity against pathogen (*Suhendar, 2023; Zhang et al., 2024*) and as an antioxidant agent (*Adi, 2022; Bamba et al., 2024*). *Radiati et al (2022)* reported that the addition of *S. cerevisiae* ATCC 36858 by 1% (w/v) into goat's milk kefir formulations resulted in antimicrobial activity of 25, 28, and 22 mm zone inhibition against *Eschericia coli, Streptococcus typhi*, and *Klebsiella pneumoniae* respectively, β −galactosidase of 120.33 U/g protein, and antioxidant activities of 72% DPPH inhibition. They argued that β −galactosidase from *S. cerevisiae* convert milk fat into short-chain fatty acids that are useful material for the host to improve probiotic ice cream texture acceptance. Based on this description, the use of *S. cerevisiae* ATCC 36858 was thought to produce kefir ice cream. The purpose of this research was to determine the character of kefir ice cream enrichment with *S. cerevisiae* ATCC 36858 in various concentration. The novelty of this study is *S. cerevisiae* population reconstruction to produce kefir ice cream that has good overall acceptance.

MATERIALS AND METHODS

MATERIAL

S. cerevisiae ATCC 36858 (10^7 CFU/g) (0; 0.33; 0.66; 1 % w/v) and kefir grains (*Lactobacillus plantarum, L. cremoris, Streptococcus cremoris*, and *Saccharomyces* sp.) were obtained from the Animal Product Technology Laboratory, Animal Science Faculty of Universitas Brawijaya. Commercial goat's milk was obtained from local farmers, Malang, Indonesia. Glucose as sweetener and mono-acyl glycerol (MAG) as emulsifier were obtained from Subur Kimia Jaya, Indonesia. Egg yolk as stabilizer and salt (NaCl) as flavor agent were obtained from traditional market, Malang, Indonesia.

GOAT'S MILK KEFIR PREPARATION

Goat milk kefir was made based on the method of *Radiati et al (2022)* with slight modification by adding 0; 0.33; 0.66; and 1% (w/v) *S. cerevisiae* ATCC 36858 and 4% (w/v) kefir grain into goat's milk. The mixture was put into a non-plastic glass jar with a volume of 1000 ml. The fermentation process was carried out for 48 hours at room temperature. After that, the mixture was filtered using a fine sieve to separate grains from the liquid. The liquid was stored in a 4°C refrigerator for temporary storage or directly used to produce kefir ice cream.

KEFIR ICE CREAM PRODUCTION

Kefir ice cream was produced using a protocol from *Zoumpopoulou et al (2021)* with slight modification by putting kefir goat's milk, MAG, glucose, salt, and egg yolk into a bowl to make ICM. The dosage of ingredients was listed in Table 1. The ICM was homogenized, then put into ice cream machine (GEA ICE – 1530) for 45 minutes. The thickened ICM was taken using a spatula and stored into an airtight plastic container to be stored in a freezer (-20°C) for 4 hours before being used for research. Stirring was done every 4 hours to keep kefir ice cream's viscosity in good condition.

Table 1

Formulation of kefir ice cream enrichment with *S. cerevisiae* ATCC 36858 in various concentration					
Ingredients		T0 (control)	T1	T2	T3
Goat's milk	**[mL]**	750	750	750	750
MAG	**[g]**	3	3	3	3
Glucose	**[mL]**	120	120	120	120
Egg yolk	**[mL]**	300	300	300	300
Kefir grains	**[g]**	30	30	30	30
Salt	**[g]**	2	2	2	2
***S. cerevisiae* ATCC 36858**	**[g]**	0	2.5	5	7.5

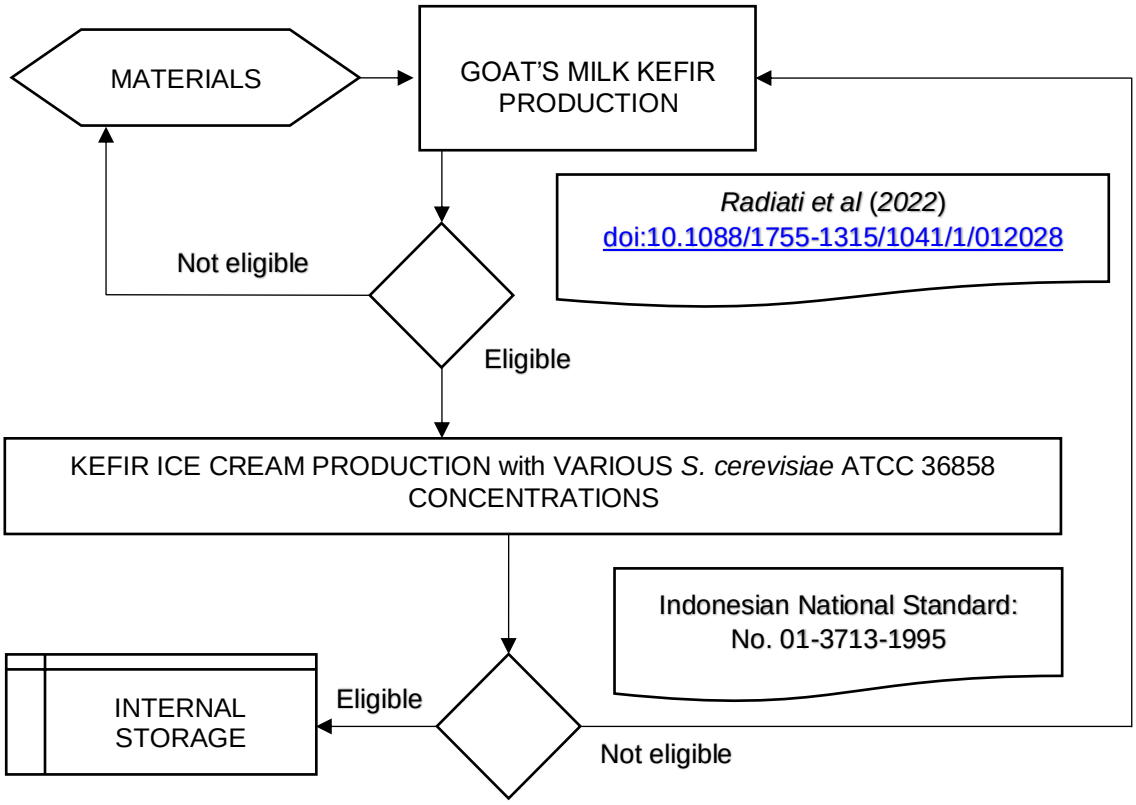


Fig. 1 – Producing of kefir ice cream

VIABILITY of CULTURE

Enumeration was carried out based on protocol by *Arief et al (2024)* with slight modification. The viability of culture (LAB and yeast) inside kefir ice cream products were determined throughout storage time. LAB and yeast were incubated in Man Rogosa Sharpe (MRS) broth (t=24 hours; T=37°C) and yeast extract, peptone, dextrose (YPD) broth (t=48 hours; T=37°C), respectively. 0.9% NaCl solution serial dilution of kefir ice cream was created and homogenized for 2 min. Serial dilutions were prepared and 100 μL from each dilution was spread to MRS and YPD agar plates for LAB and yeast, respectively. The viability of LAB and yeast were calculated using Equation 1.

$$Viability\ of\ culture = \frac{a}{((1 \times b) + (0{,}1 \times c) \times d)} \quad (1)$$

Where:

a =*Calculated colony*
b =*Sum of Petri dish on the first dilution*
c =*Sum of petridish on the next dillution*
d =*First dilution*

PHYSICO-CHEMICAL FEATURES DETERMINATION

Kefir ice cream was melted at room temperature and characterized for pH by potentiometric method (Digimed DM20), titratable acidity by titration method and expressed as a lactic acid percentage, viscosity by viscometer (NDJ-8S) at 30 rpm for 30 seconds, total protein by Kjeldhal method, fat by Gerber method, and ethanol content by titration method based on Indonesian National Standard 01-2891-1992 (*Indonesian National Standardization Agency 1992*).

MELTING FEATURES DETERMINATION

Melting testing was carried out based on protocol by *Muse and Hartel (2004)* with slight modifications. A sample of kefir ice cream blocks (6cm×6cm×3cm) with mass ±100 gr. The initial temperature of ice cream was -17°C. Then they were placed on a wire screen. The sample was placed in a controlled temperature chamber at 25°C. The dripped volume was recorded after 30 minutes, 60 minutes, and 90 minutes and calculated as percentage drainage using Equation 2.

$$\frac{a}{b} \times 100\% \quad (2)$$

where: a = Mass of melted ice cream; b = Mass of frozen ice cream

OVERRUN FEATURES DETERMINATION

Overrun calculations were carried out based on *Kozłowicz et al. (2019)* with slight modifications. The mass of ICM was weighed as and recorded as "a". The mass of kefir ice cream was weighed and recorded as "b". Mass weighing was carried out according to the theory of the equation that 1 gram is equivalent to 1 cm^3. Overrun was calculated using Equation 3.

$$\frac{b-a}{a} \times 100\% \quad (3)$$

where:

a = Mass of ICM; b = Volume of kefir ice cream

COLOR FEATURES DETERMINATION

Color determination was carried out based on protocol by *Kozłowicz et al. (2019)* with slight modification. The color values of probiotic ice cream in terms of L*, a*, and b* values were determined using a CR-400 ChromaMeter (Konica Minolta, Japan) instrument.

MORPHOLOGY AND MINERAL CONTENT FEATURES DETERMINATION

Kefir ice cream was melted at room temperature and were characterized for morphology based on *Florczuk et al. (2022)* using field emission scanning electron microscopy (FESEM) – Energy Dispersive Spectroscopy (EDS) from the FESEM Thermo Scientific Quattro with EDS detector was used to capture the surface morphology of kefir ice cream. The same engine was used to calculate the weight percentages of components.

SENSORY FEATURES

Sensory evaluation was carried out based on protocol by *Hanafi et al. (2022)* with slight modification. In this analysis, 30 panelists (15 men dan 15 woman) present their personal responses written using a hedonic scale on 1 to 5 (1: very bad, 2: dislike, 3: not too bad, 4: good and 5: very good) during the education period held to provide knowledge about probiotic ice cream in two hours meeting. Ethical clearance regarding the use of panelists based on protocol by Guidelines for the Code of Ethics for Research and Community Service Universitas Brawijaya.

EXPERIMENTAL DESIGN AND DATA ANALYZES

The experimental design used in this study was a Completely Randomized Design (CRD) with four treatments 0%; 0.33%; 0.66%; 1% (w/v) *S. cerevisiae* ATCC 36858 addition and four repetitions respectively. Kefir ice cream's formulation can be seen in Table 1. Statistical analysis was performed for all measurement data using Microsoft Excel with One Way Analysis of Variance (ANOVA). The differences between treatments were tested further using Duncan's Multiple Range Test (DMRT) analysis with a significance level of 0.05.

BEST TREATMENT DETERMINATION

The analysis for determining the best formulation was carried out by the method of De Garmo (*Alfadila et al. 2020*). Determining the best treatment begins with grouping all observation parameters and scoring. Then calculate the weight value (WV) to determine the treatment value, the best value, and the worst value. Then calculated the effectiveness value (EV) with the Equation 4.

$$\frac{(a-b)}{(c-b)} \quad (4)$$

where:

a = Treatment value; b = Worst value; c = Best value

After that, the calculation of the product value for each observation parameter was carried out using the Equation 5.

$$a \times b \quad (5)$$

where:

a = *Effectiveness value;* b = *Weight value*

RESULTS

Culture Viability of Kefir Ice Cream

The initial count of LAB viability was $2.35x10^7$ CFU/g for control and all treatments. The results showed that the control and all treatments decreased the viability of LAB by $1x10^4$ and $5x10^5$ CFU/g on day 1st to day 7th; $4x10^5$ and $1x10^6$ CFU/g on day 14th; $1x10^6$ and $4x10^5$ CFU/g on day 21st to day 28th respectively. While the initial count of yeast viability for control and all treatments was $9.11x10^4$; $9.13x10^4$; $9.15x10^4$; $9.20x10^4$ CFU/g respectively. The results showed that control and all treatments decreased the viability of yeast by $1x10^4$ and $2x10^3$ CFU/g on day 1st; $2x10^3$ CFU/g on day 7th; $1x10^4$ CFU/g on day 14th; $2x10^4$ CFU/g on day 21st to day 28th for control and all treatment respectively.

Kefir grains used in this study contained *L. plantarum, L. cremoris, S. cremoris*, and *Saccharomyces* sp as much as 4% (w/v). Meanwhile, the treatment used in this study was the addition of *S. cerevisiae* ATCC 36858 strains as much as 0.33, 0.66, and 1% (w/v). The probiotic community was thought to have a positive impact on human health. *Akalin et al (2018)* argue that *Lactobacillus* sp is classified as LAB which can help maintain human digestive health. *Bamba et al (2024)* argue that *Saccharomyces* sp is a yeast that can establish mutualism symbiosis with LAB. *Arief et al (2024)* reported that the viability of LAB and yeast during ice cream production showed a good viability rate. Unfortunately, there were several factors that can reduce this number when entering the storage stage. Probiotic products should contain viable count of microorganisms at numbers ranging from 10^6 to 10^9 CFU/g during their shelf-life (*Bullock & Gruen, 2023*).

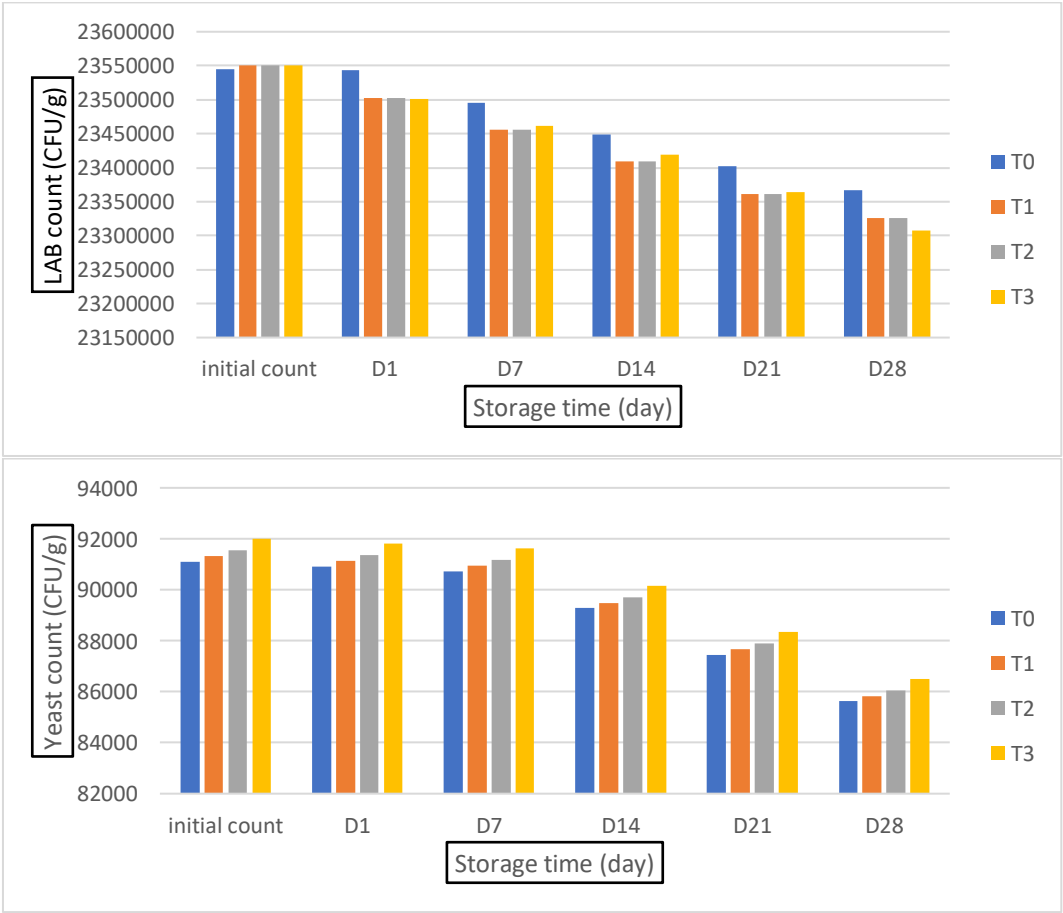


Fig. 2 - Viability of culture (LAB and yeast) in kefir ice cream product during 28 days storage period

Fig. 2 shows the viability of LAB and yeast in kefir ice cream product. For the record, the calculation of viable count of *S. cerevisiae* ATCC 36858 was put together with *Saccharomyces* sp. During the ice cream production, LAB and yeast were inoculated at level of $2.354529 \times 10^7$and 9.1100×10^4 CFU/g respectively. The initial count results showed that the decrease in viability of LAB was higher than that of yeast (Fig. 2). Many LAB suffer a rapid loss of their viability if stored at temperatures below -17°C (*Goktas et al., 2022*). This could be due to the yeasts which are more adaptable to low temperature than bacteria.

As expected, the probiotic numbers in ice cream products were in a decrement trend during the storage time but importantly none of the ice cream products harbored probiotics below 8.5634×10^4 CFU/g. This level was within the suitable level to be classified as probiotic products based on Codex Alimentarius. The number of LAB and yeast is at least $10^6 – 10^9$ and 10^4 CFU/g, respectively (*Codex, 2018*).

The decrement of viable culture number was probably because of the freezing stage which caused some damage to cells and finally they died (*Corradini et al., 2014*). *Cruz et al (2022)* argued that decrement of viable LAB and yeast in the product may be related to competition. The decrease of viable counts during storage time could be caused by the low temperature (- 20°C) storage condition. Following the D-28th of storage period, viable counts of LAB and yeast were at 2.3308100×10^7 and 8.5634×10^4 CFU/g levels, respectively. This result can be critical for the numbers because the LAB has decreased drastically as much as 1×10^6 CFU/g in D-14th storage, while yeast experienced a drastic decrease as much as 2×10^4 CFU/g on D-21st storage. These findings suggest that the product storage should not be more than 28 days.

Physico-chemical Features of Kefir Ice Cream

There was a significant increase in acidity, ethanol and viscosity, but this was not the case with protein inside kefir ice cream (Table 2). The difference that causes an increase in acidity and ethanol appears significantly in T0 with T1. There was also a significant decrease in pH (D-1), pH (D-28), overrun, melting in 30 minutes, 60 minutes, and 90 minutes, but this did not occur in fat inside kefir ice cream (Table 2). The difference that causes a decrease in pH D-1st and D-28th appears significantly in T2 with T1 and T1 with control. While against overrun there was a significant difference in T1 with control. Unfortunately, there was no significant difference between treatments despite a decrease in melting in 30; 60; and 90 minutes.

Table 2

Effect of various S. cerevisiae ATCC 36858 concentrations on physico-chemical features of kefir ice cream

Attributes			Concentration of *S. cerevisiae* ATCC 36858		
		Control (T0)	T1	T2	T3
pH (D-1st)	[pH]	6.71 ± 0.03c	6.34 ± 0.03b	6.29 ± 0.03a	6.25 ± 0.02a
pH (D-28th)	[pH]	6.68 ± 0.02c	6.27 ± 0.02b	6.23 ± 0.03a	6.21 ± 0.01a
Acidity	[%]	0.18 ± 0.00a	0.34 ± 0.08b	0.37 ± 0.02b	0.38 ± 0.00b
Fat	[%]	3.20 ± 0.01	3.15 ± 0.13	3.13 ± 0.13	3.11 ± 0.12
Protein	[%]	3.35 ± 0.21	3.65 ± 0.36	3.68 ± 0.36	3.69 ± 0.34
Ethanol	[%]	0.50 ± 0.03a	0.69 ± 0.06b	0.76 ± 0.07b	0.88 ± 0.08b
Overrun	[%]	31.13 ± 0.22b	27.05 ± 0.82a	27.00 ± 0.82a	26.99 ± 0.85a
Melting in 30 min	[%]	27 ± 0.05a	25 ± 0.06a	19 ± 0.06a	14 ± 0.06a
Melting in 60 min	[%]	39 ± 0.05a	34 ± 0.06a	27 ± 0.06a	25 ± 0.06a
Melting in 90 min	[%]	87 ± 0.05a	81 ± 0.06a	77 ± 0.06a	69 ± 0.06a
Viscosity	[m.Pa]	65.15 ± 6.12a	70.08 ± 6.19a	81.93 ± 6.26b	83.29 ± 6.33b

Means in the same row with different superscript differ significantly ($p<0.05$)

Kozłowicz et al (2019) argued that LAB and yeast as kefir grains are enclosed in a polysaccharide known as kefiran and protein matrix. *McGhee et al (2015)* argued that the microbial fermentation of kefir product produces organic compounds such as lactic acid and ethanol. Under favorable conditions, the fermentation process begins and leads to the increased number of their viability and produces various metabolites at the end of the process. Therefore, in making this kefir ice cream, nutritional conditioning was well maintained to meet the life needs of LAB and yeast by adding *S. cerevisiae* ATCC 36858 to the formula. *Radiati et al (2022)* reported that *S. cerevisiae* ATCC 36858 in grain kefir complex improved bioactive compound of goat's milk kefir and simultaneously gave some improvement by producing antimicrobial activity, antioxidant, and β − galactosidase enzyme. Besides that, this enzyme converts milk fat into short-chain fatty acids that are useful material for the host.

Bamba et al (2023) reported that *S. cerevisiae* produced glycerol and ethanol around 1.1 and 5.4 g/L at 48 h of cultures respectively. In addition, *Khademi et al (2022)* reported that *S. cerevisiae* could lower the pH value continuously during culture and reached a value of 4.4 pH. Furthermore, *Gut et al (2022)* reported that from 5.5 to 6.5 pH led to the increased survival of LAB and yeast during storage. The ice cream matrix could become a good vehicle for LAB and yeast culture which contained milk protein and fat.

High fat content can provide support considerably higher viability of probiotic strains during production and storage (*Zoumpopoulou et al 2021*).

The fat content of kefir ice cream product changed between 3.20% and 3.11%. In the particular case, this implies overcoming intrinsic hurdles such as the beating step, where air is incorporated known as overrun and storage under freezing temperatures. *Dutra et al (2015)* stated similarly that in general, addition of LAB and yeast resulted in lower overrun levels in comparison with control.

The overrun defines the structure of the final product, since the presence of air gives the kefir ice cream an agreeable creamy texture. *Jardines et al (2020)* reported that commercially, the overrun of all the probiotic ice cream was low, which were indicated to be between 50% and 80%. An increase in *S. cerevisiae* ATCC 36858 used decreased the overrun of kefir ice cream. A higher overrun led to a slower melting because air cells acted as an isolator medium (*Deosarkar et al., 2016*). Melting rate feature affects the formation of hardness texture of the final product. The increased proportion of *S. cerevisiae* ATCC 36858 is able to improve viscosity while decreasing the melting of kefir ice cream.

The melting was also associated with the overrun. These current findings show that increasing the amount of *S. cerevisiae* ATCC 36858 reduces overrun, causing kefir ice cream to melt more slowly. *Akbari et al (2019)* reported that low air cell incorporation might function as an insulator inside the kefir ice cream structure. Whereas, *Arief et al (2023)* reported that the addition of partially mixed fat derived from egg yolk was able to form a fat bond which could stabilize the air bubble and foam in the ice cream structure, which subsequently reduced the melting around 80 – 90 % when the block of kefir ice cream was allowed to melt at room temperature for 2 hours.

Viscosity feature is the resistance of fluids to changes in movement relative to each other (*Fitri et al., 2020*). The viscosity value will appear when the molecules in the fluid friction against each other. The increase in the viscosity of kefir ice cream containing *S. cerevisiae* ATCC 36858 was probably due to the high-water retention of insoluble materials based on their low soluble content. *Alfadila et al (2020)* argued that the increment indicated the significant effect of LAB on texture of the ice cream in viscosity. *Popescu et al (2021)* argued that enhanced viscosity can be achieved with the interaction of milk protein or by the interaction between soluble and insoluble compound.

Color Feature of Kefir Ice Cream

The results showed that the addition of *S. cerevisiae* ATCC 36858 with various concentrations was able to produce a brighter product than the control (Table 3). This was because *S. cerevisiae* ATCC 36858 produces products that tend to be greenish and yellowish significantly. Based on the DMRT test, it was known that the color tendency of T1 and T2 products was bright yellowish and bright greenish respectively. The duration of product storage for 28 days was able to significantly reduce product brightness (Table 3). DMRT tests show that the difference between treatments that cause a decrease in brightness tends to be greenish occurs insignificantly, while the yellowish color turns bluish. DMRT tests show that there was a significant difference in each treatment of yellow to bluish color change.

Table 3

Effect of various S. cerevisiae ATCC 36858 concentrations on color features of kefir ice cream day 1st and day 28th

Sample	D-1st			D-28th		
	L*	a*	b*	L*	a*	b*
Control (T0)	84.47±0.32^{a}	-5.95±0.03^{a}	11.45±0.05^{a}	83.14±0.43^{a}	-6.02±0.03^{a}	11.31±0.05^{c}
T1	86.84±0.33^{b}	-5.91±0.03^{b}	12.36±0.06^{b}	84.87±0.65^{a}	-5.74±0.02^{a}	10.78±0.05^{b}
T2	87.62±0.33^{c}	-5.84±0.03^{c}	12.21±0.06^{b}	84.31±0.43^{a}	-5.67±0.02^{a}	10.65±0.05^{b}
T3	88.41±0.34^{c}	-5.77±0.02^{c}	12.06±0.06^{b}	83.30±0.43^{a}	-5.60±0.02^{a}	10.52±0.05^{a}

Means in the same row with different superscript differ significantly ($p<0.05$). L* values show the light intensity between 0 – 100, a* values show the redness (+) to green (-) and b* values show yellowness (+) to blueness (-) color.

Color feature of kefir ice cream is one of the most critical quality indicators accepted by panelist. L* (brightness) of kefir ice cream changed between 84.47 and 88.41 observed for all treatment. The addition of 1% *S. cerevisiae* ATCC 36858 resulted in significantly higher L* compared to control. After 28 days of storage time, differences in L* value of kefir ice cream were found to be significant. *Sarwar et al (2021)* reported that with the exception of yellowness, all kefir ice cream products prepared with fat replacers had significantly better appearance acceptance than control. In terms of a* (redness) dan b*(yellowness) of kefir ice cream, a* values changed in the range of – 5.95 to – 5.77 whereas b* values product altered in the range of 11.45 to 12.06. An improvement in a* values of all kefir ice cream products containing *S. cerevisiae* ATCC 36858 was observed after 28 days of storage time whereas a decrement was observed for a* and b* values.

Structure and mineral of Kefir Ice Cream

FESEM results showed that the particles were formed from agglomerations of various sizes (Fig. 3). The particles seemed to be trying to form large spheres. At 100X magnification, these tiny particles were not clearly visible. Fig 3 shows that the addition of *S. cerevisiae* ATCC 36858 to the formula affects the agglomeration process. The particles in the treatment showed increased agglomeration activity compared to the control. The agglomerations formed were observed using 100X magnification to obtain overall results and continued using 500X and 1,000X magnifications. The results of the enlargement showed that the appearance of voids gradually began to fade as the concentration of *S. cerevisiae* ATCC 36858 increased.

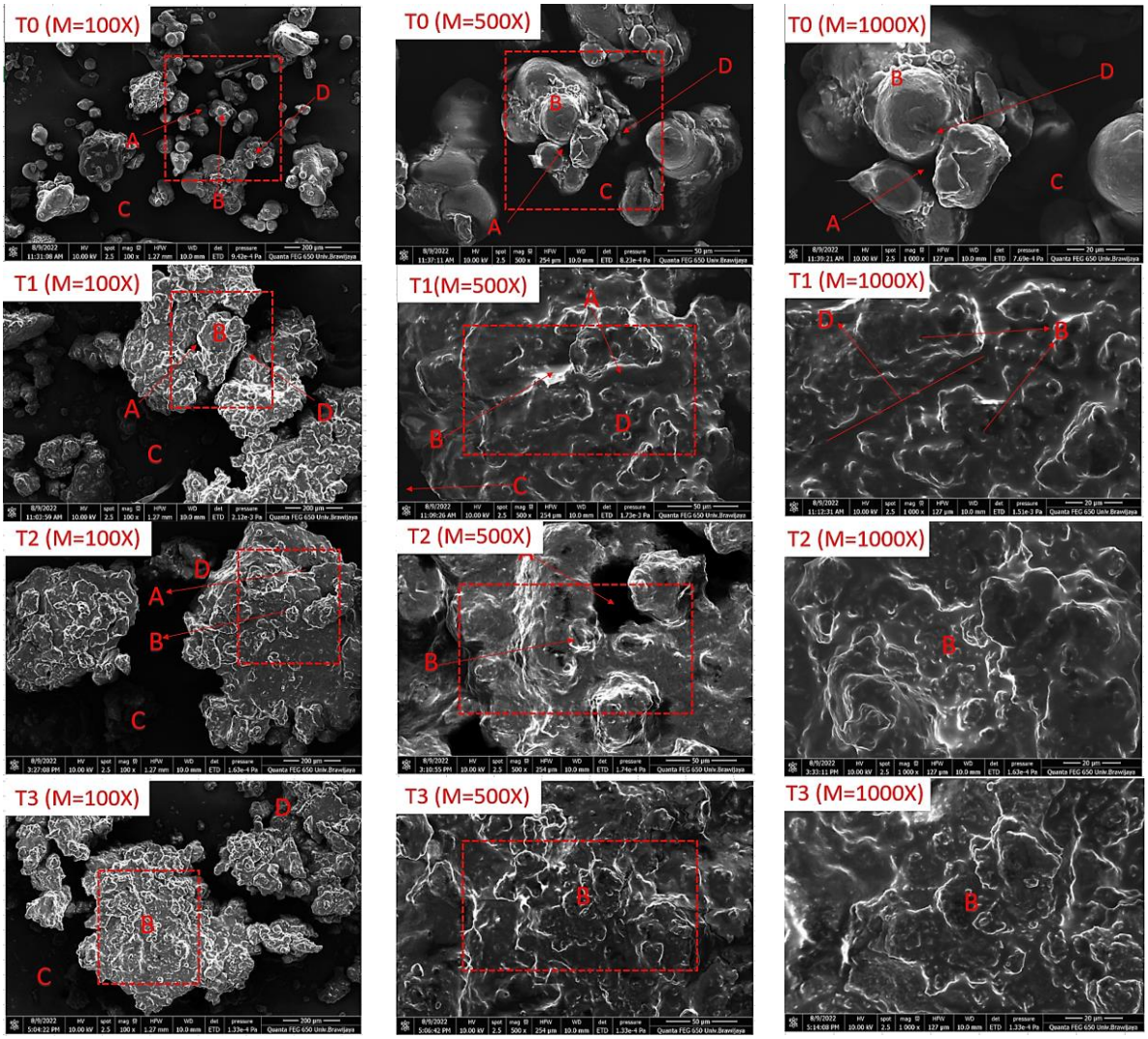
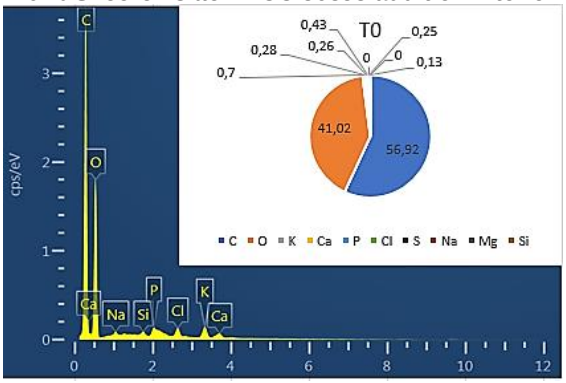


Fig. 3 - The FESEM result of enrichment kefir ice cream with *S. cerevisiae* ATCC 36858

Appearance of kefir ice cream enrichment with S. cerevisiae ATCC 36858 in various concentration based on FESEM-EDS overview. A – Voids that thwart the formation of agglomerations, B – Fat droplets attempt to attract particles around them to unite, C – Water as a solvent has the potential to dissolve the particles formed to become a homogeneous solution, D – Fat droplets attempt to form agglomerations with the help of casein micelles. Agglomeration was formed if no voids were found in them. An increase in the concentration of S. cerevisiae ATCC 36858 of at least 0.33% was able to minimize the appearance of voids. This was evidenced by the disappearance of voids in T1 and T2 which were observed using 1,000X magnification. This was further strengthened by the absence of voids in the T3 formula that was observed using 500X magnification.

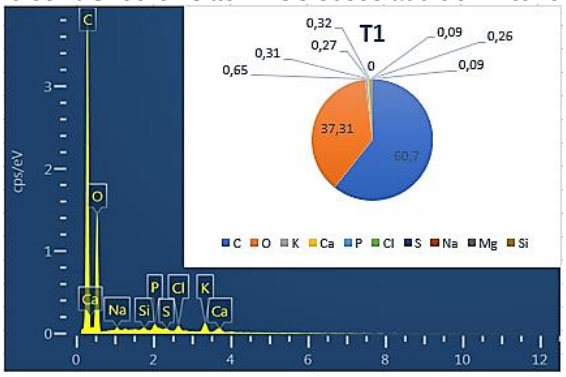
FESEM + EDS results showed that the addition of *S. cerevisiae* ATCC 36858 also affected the mineral content of kefir ice cream (Fig. 4). The mineral levels contained in kefir ice cream were C (56.92 – 65.43%), O (30.37 – 41.02%), K (0.70 – 1.04%), Cl (0.43 – 0.73%), Ca (0.28 – 0.96%), P (0.26 – 0.82%), Na (0.25 – 0.36%), S (0.00 – 0.26%), Mg (0.00 – 0.22%), and Si (0.00 – 0.13%). These micronutrients come from the ingredients used to produce kefir ice cream such us, goat's milk (Ca, Mg, P, and K), glucose (C and O), egg yolk (P, Ca, and Mg), and salt (Na and Cl). Based on result, it was known that kefir ice cream has an average of C (61.85 ± 3.86), O (34.90 ± 5.15), K (0.88 ± 0.24), Ca (0.62 ± 0.38), P (0.55 ± 0.33), Cl (0.59 ± 0.26), S (0.15 ± 0.13), Na (0.27 ± 0.05), Mg (0.09 ± 0.11), and Si (0.05 ± 0.06).

0 % *S. cerevisiae* ATCC 36858 addition into formula



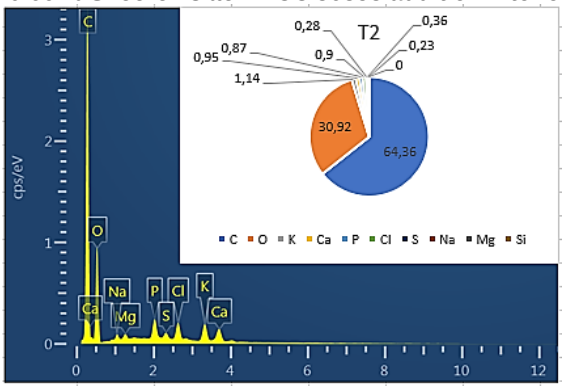
Element	Weight (%)
C	56.92
O	41.02
K	0.70
Ca	0.28
P	0.26
Cl	0.43
S	0.00
Na	0.25
Mg	0.00
Si	0.13

0.33 % *S. cerevisiae* ATCC 36858 addition into formula



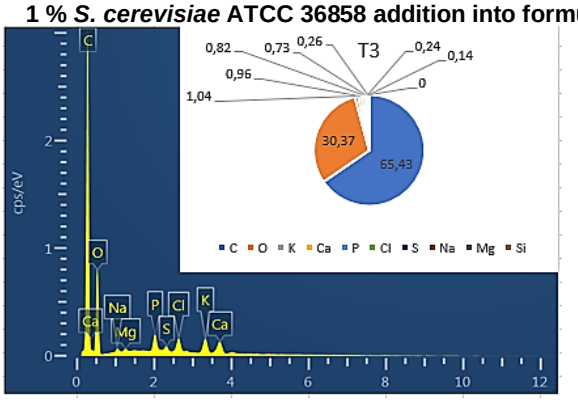
Element	Weight (%)
C	60.7
O	37.31
K	0.65
Ca	0.31
P	0.27
Cl	0.32
S	0.09
Na	0.26
Mg	0.00
Si	0.09

0.66 % *S. cerevisiae* ATCC 36858 addition into formula



Element	Weight (%)
C	64.36
O	30.92
K	1.14
Ca	0.95
P	0.87
Cl	0.90
S	0.28
Na	0.36
Mg	0.23
Si	0.00

1 % *S. cerevisiae* ATCC 36858 addition into formula



Element	Weight (%)
C	65.43
O	30.37
K	1.04
Ca	0.96
P	0.82
Cl	0.73
S	0.26
Na	0.24
Mg	0.14
Si	0.00

Fig. 4 - Mineral contents of kefir ice cream enrichment with *S. cerevisiae* ATCC 36858 in various concentration for each treatment assessment result by FESEM-EDS

The difference in micronutrient content of each treatment was thought to be caused by LAB and yeast activities. Ali *et al* (2022) report that *S. cerevisiae* is known to be an obligate aerobe so they require oxygen for cellular respiration. Georgiev *et al* (2024) report that glucose is a monosaccharide that easily fermented by *S. cerevisiae* as a food source to produce phytase enzymes and cause increased phosphorus levels during the milk fermentation process. *Casertano et al. (2022)* argued that micronutrient such as mineral and vitamin should not result in disagreeable flavors negatively affecting the panelists acceptance.

Salari et al. (2021) argued that macro minerals such as Ca, Mg, Na, and K are essential for controlling blood pressure, while micro minerals such as Si are able to help repair cell damage. Unfortunately, although kefir ice cream was made from goat's milk, no Zn content was found in them. *Fernandes et al. (2022)* argued that Zn is a mineral derived from milk-based foods that functions as an antioxidant and strengthens the body's immune system. Even so, *Umam et al. (2021)* reported that goat's milk kefir supplemented with *S. cerevisiae* ATCC 36858 has good antioxidants and peptide bonds to support the consumer's immune system. However, excess minerals are also not good for the body. *Warren and Hartel (2018)* argued that excess consumption of Na causes poisoning, even in acute circumstances can cause hypertension. *Karim and Aider (2022)* also argued that excess K causes side effects such as stomach ache and heart attack. Therefore, consumption of kefir ice cream needs to be maintained even in order to obtain optimal health effects.

Sensory Features of Kefir Ice Cream

Hedonic tests show that the addition of *S. cerevisiae* ATCC 36858 with various concentrations can significantly affect flavor, melting, texture, color, and overall acceptance (Table 4). Unfortunately, the results of the DMRT test showed that there was no significant difference between the treatment of all sensory features. However, each product has its own acceptance value for each treatment (Fig. 5). The most favorable products by the panelists were T2 (flavor) and T3 (melting, texture, and color). Whereas the least favorable ones were T3 (flavor) and T0 (melting, texture, and color). While the favorite product category was T2 because it has the highest overall acceptance value.

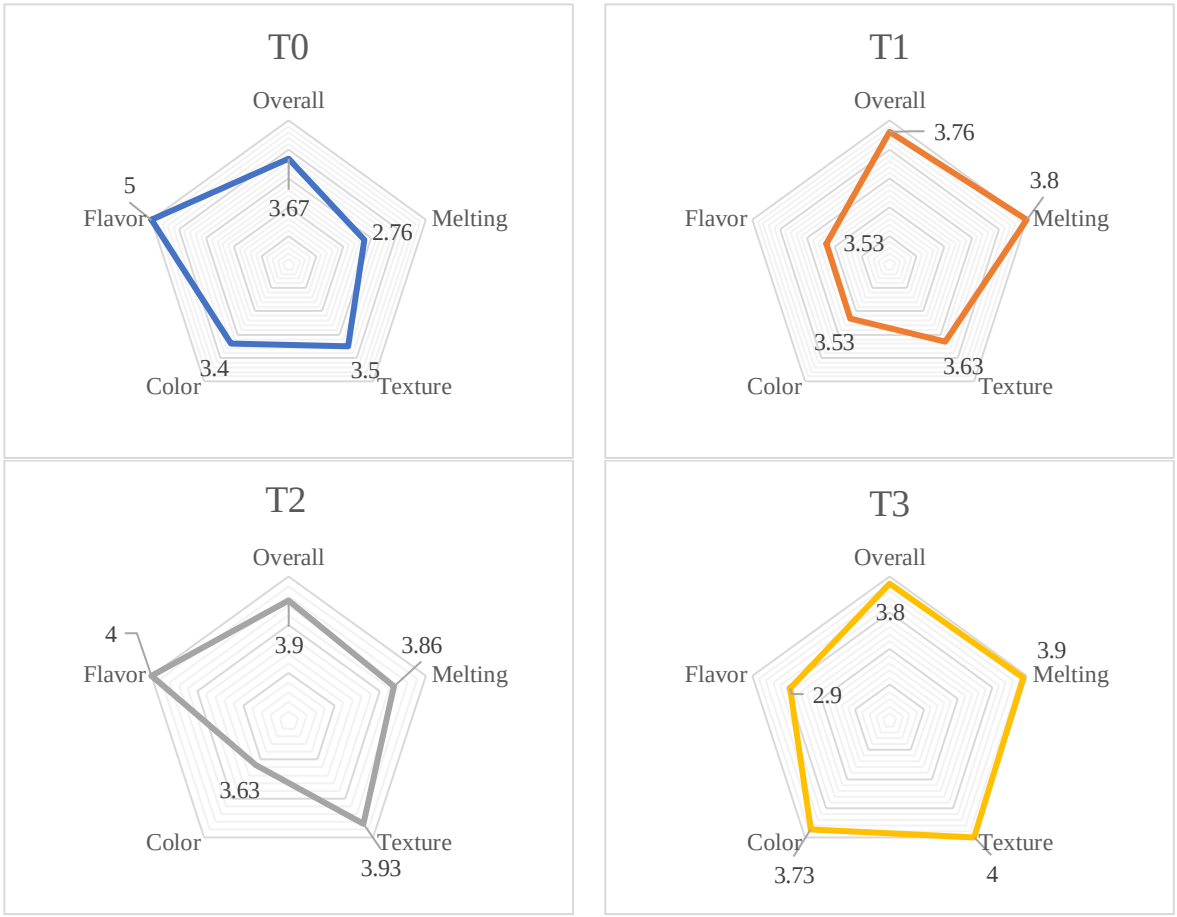


Fig. 5 - The hedonic assessment result of enrichment kefir ice cream with *S. cerevisiae* ATCC 36858.

As a general rule, the addition of *S. cerevisiae* ATCC 36858 into a kefir ice cream implies the need to assure the viability of LAB and yeast without altering its sensory features. Some physico-chemical features affect sensory features. *Zhang et al (2024)* reported that the differences in the overrun step of kefir ice cream were related to the acidifying capacity of the LAB and yeast cultures, which affects the origin protein. *Khademi et al (2022)* argued that the sensory feature of textural appearance corresponded to whether a sample cut smoothly or crumbled when cut into with a spoon. *Gut et al (2022)* reported that graininess was related to whether the surface had the typical appearance of a full fat product or icy appearance of fat-free ice cream.

Table 4

Effect of various *S. cerevisiae* ATCC 36858 concentrations on sensory features of kefir ice cream				
Attributes	**Concentration of *S. cerevisiae* ATCC 36858**			
	Control (T0)	**T1**	**T2**	**T3**
Flavor	3.67±0.95[a]	3.53±1.13[a]	4.00±0.74[a]	2.90±0.71[a]
Melting	2.76±0.93[a]	3.80±0.61[a]	3.86±0.77[a]	3.90±0.80[a]
Texture	3.50±0.93[a]	3.63±0.76[a]	3.93±0.78[a]	4.00±0.74[a]
Color	3.40±0.77[a]	3.53±0.50[a]	3.63±0.66[a]	3.73±0.82[a]
Overall	3.23±0.81[a]	3.76±0.62[a]	3.90±0.80[a]	3.80±0.80[a]
Means in the same row with different superscript differ significantly (p<0.05)				

The appearance term air holes described whether or not discrete air pockets were observed as the panelists scraped the surface of the kefir ice cream with a spoon. The appearance term stickiness referred to product cohesion. Fig. 3 shows that the particles were formed from agglomerations of various sizes, due to the addition of *S. cerevisiae* ATCC 36858. The particles in the treatment showed the increased agglomeration activity compared to the control. Void spaces within the casein matrix were spherical, showing that they were formed by fat droplets interrupting the aggregation of casein micelles (*Yuzhen et al., 2023*). In general, there was only fat droplet per void space. The casein micelles thus were surrounded by each fat droplet separating it from the others. An increase in the concentration of *S. cerevisiae* of at least 0.33% was able to minimize the appearance of voids (Fig.3). These evidenced by the disappearance of voids in T1 and T2 observed using 1,000X magnification. This was further strengthened by the absence of voids in the T3 formula which observed using 500X magnification.

Table 5

Attributes	**Treatment**				**Best Value**	**Worst Value**	**Differ**
	T0	**T1**	**T2**	**T3**			
Flavor	5.00	6.00	6.16	6.00	6.16	5.00	1.16
Melting	5.16	6.00	6.36	6.26	6.26	5.16	1.10
Texture	5.23	6.00	6.36	6.40	6.40	5.23	1.16
Color	5.03	5.23	5.33	5.43	5.43	5.03	0.40
Overall	5.63	6.10	6.40	6.20	6.40	5.63	0.77

Attributes	**WV**	**Treatment**							
		T0		**T1**		**T2**		**T3**	
		EV	**PV**	**EV**	**PV**	**EV**	**PV**	**EV**	**PV**
Flavor	0.26	0.00	0.00	0.85	0.22	1.00	0.26	0.85	0.22
Melting	0.13	0.00	0.00	0.81	0.10	1.03	0.13	1.00	0.13
Texture	0.20	0.00	0.00	0.68	0.13	0.97	0.19	1.00	0.20
Color	0.06	0.00	0.00	0.50	0.03	0.75	0.05	1.00	0.06
Overall	0.33	0.00	0.00	0.60	0.20	1.00	0.33	0.73	0.24
Total	1.00	0.00	0.00	3.44	0.68	4.75	0.96	4.58	0.85

Note:

WV	=	Weight value
EV	=	Effectivity value
PV	=	Product value, Best treatment was determined by the highest score of PV

The results showed that kefir ice cream produced with LAB and yeast cultures only (control), present a less intense aroma and kefir flavor than the product prepared with *S. cerevisiae* ATCC 36858 cultures (Table 5). The addition of *S. cerevisiae* ATCC 36858 does not modify the sensory features of the control intensely. Unfortunately, T3 was thought to have an off flavor due to too much addition of *S. cerevisiae* ATCC 36858, resulting in lactic acid and acidity extremely. The authors suggested that the use of *S. cerevisiae* ATCC 36858 was only 0.66% because there was no significant difference between T2 and T3, mainly with respect to the product acidity and sensory features. Overall, T2 had the best value compared to other treatments. T2 has sufficient pH, acidity, and ethanol compared to other treatments so that it has the right flavor. T2 has enough overrun and viscosity to slow down the melting rate to produce textures that were quite desirable. In the present study, the best treatment of kefir ice cream was determined by de Garmo method. The score of the T0, T1, T2, and T3 product value was 0.00; 0.68; 0.96; and 0.85 respectively. This result shows that the optimum concentration of *S. cerevisiae* ATCC for use was 5 g per 750 ml (T2) as the best treatment.

CONCLUSIONS

The addition of *S. cerevisiae* ATCC 36858 with a concentration of 0.66% was the best treatment to obtain the highest overall acceptance value. The addition of more than 0.66% makes kefir ice cream a very acidic product and the flavor was not liked by panelists. An addition of less than 0.66% was still less to get the sensation of flavor and texture from kefir ice cream products. In addition, LAB and yeast in kefir ice cream can survive for 28 days at – 20^{o}C.

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