



Yeast Detection in Mango Flavored Kefir from Small Scale Manufacture in Semarang, Central Java, Indonesia

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Abstract

This research was done to detect total yeast on small scale kefir industry in Semarang, Central Java, Indonesia. The total plate count method was used to analyze total yeast in mango flavored kefir. This research used mango flavored kefir since this products was the top demand by consumer among kefir flavored products that was produced in this manufacture. Kefir from manufacture was transferred to laboratory using container box to provide buffer in the temperature decrease. As result, total yeast in mango flavored kefir was $1,21 \pm 0,06 \times 10^5$ CFU/ml. This total yeast was in the range of the Standard Codex 243-2003 which required a minimum total of 10^4 CFU/ml. The 1 log unit differences should provide the awareness to manufacture to maintain kefir in low temperature to minimise the multiplication of total yeast resulting the appearance of unwanted flavor. As conclusion, the mango flavored kefir was analyzed in total yeast successfully and in the range of Standard Codex of kefir.

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Introduction

Kefir is a fermented milk originated in the Caucasus, this acidic fermented milk is slightly carbonated and present small amount of alcohol. What distinguishes kefir from the traditional yoghurt is that is made only from kefir grains, which are composed of yeast and bacteria (Otsoa *et al.*, 2006). The "traditional" way of producing kefir using raw unpasteurized, or UHT treated milk. Yeast is a microorganism belonging to the unicellular fungi that does not form mycelium (Dickinson and Schweizer, 2004). Kefir grains consist of lactic acid bacteria and yeast. *Leuconostoc* yeast forms diacetyl and candida kefir forming ethanol and CO₂ (Susilorini and Sawitri, 2005).

Total yeast in kefir can be influenced by the amount of sugar added to kefir, because sugar will be used by yeast to grow and multiply, some will be converted into metabolite products are alcohol and CO₂ (Hawusiwa *et al.*, 2015). Total yeast in kefir can also be influenced by the number of kefir grains used, because the more number of kefir grains can increase the amount

of yeast which is also a microbe present in kefir grains (Sawitri, 2011). The kefir-specific yeast play a key role in the formation of flavour and aroma. Common yeast found in kefir is *Kluyveromyces marxianus*, *Torulaspora delbrueckii*, *Saccharomyces cerevisiae*, *Candida kefir*, *Saccharomyces unisporus*, *Pichia fermentans* and *Yarrowia lipolytica* (Simova *et al.*, 2002). *Kluyveromyces marxianus* as the yeast with the highest production of lactic acid and ethanol. Lactose nonfermenting yeast are *Saccharomyces cerevisiae* and *Saccharomyces lipolytic* (Bergmann *et al.*, 2010).

Kefir manufacture in the Semarang region was found as not much in number but it was exist since last two decades. Most people preferred the flavored kefir and the most wanted products is mango flavored kefir. Therefore this research used this product to determine the total yeast in the product after it was produced freshly from manufacture. The objective of this research was determining total yeast in mango flavored kefir after the kefir was produced from manufacture. Thus, the number was then compare with the standard codex for kefir.

Materials and Methods

Materials

This study used kefir that was provided from small scale kefir industry in Semarang, Central Java. The location of manufacture is about 1.5 hour from laboratory by car. The delivery of kefir was used container ice box to maintain the temperature and directly transferred to laboratory without any further storage. Potato dextrose agar (PDA) medium, physiological NaCl were used as main medium to calculate the number of yeast. A sterile petri dish, erlenmeyer, sterile test tubes, incubator, micropipette, autoclave were used as tool in this experiments.

Obtaining Kefir

The study was conducted from November 2018 – February 2019 at the Food Chemistry and Nutrition Laboratory, Faculty of Animal and Agriculture Sciences, Diponegoro University. Kefir with the mango flavor was freshly provided from small scale kefir manufacture in Semarang without preservation. Nine bottles were obtained in every months to calculate total yeast.

Total Yeast Calculation

Yeast was detected using total plate count method (Aristya *et al.*, 2013). Potato dextrose agar was used as a medium for yeast growth using following procedure: one milliliter kefir was prepared and put into erlenmeyer containing 9 ml of physiological NaCl using sterile pipette as dilution 10^{-1} . Then, 1 ml of suspension sample was taken and transferred into a test tube containing 9 ml of physiological NaCl as a 10^{-2} dilution then continued with the same procedure until 10^{-6} dilutions were obtained. A suspension of 1 ml of the last three dilutions was applied into the two petri dish, then 15 ml of PDA medium with a temperature of 50°C was added to the petri dish in sterile condition using laminar. The incubation was applied in using upside down position at an incubator using 30°C for 48 hours. Total yeast kefir was then calculated from the appearance of colony as colony forming units per ml (CFU ml^{-1}).

Data Analysis

Total yeast was calculated as CFU/ml and duplicate data was conducted. Data were presented as $\text{mean} \pm \text{standard deviation}$ and after received, then compared with standard Codex Stan 243-2003 (FAO, 2003; Codex Alimentarius Commission, 2003).

Results and Discussion

Total yeast in mango flavored kefir was $1,21 \pm 0,06 \times 10^5$ CFU/ml , similar to the finding of previous level, ie. 10^5 (Irigoyen *et al.*, 2005) This total yeast was in the range of the standard Codex Stan 243-2003 which requires a minimum total of yeast as much as 10^4 CFU/ml . The addition of sugar in kefir should not be more than 40% in order to control the growth of yeast resulting in the optimum taste and acidity (Hawusiwa *et al.*, 2015). Kefir is generally stored in a refrigerator where cold temperature may reduce the microorganism growth rate in kefir, then it may contribute to the development of pH, titratable acidity and taste of the product (Setyawardani and Sumarmono, 2015).

Proper treatment of the kefir is an important step in producing good quality product (Surono and Hasono, 2011). Kefir should be stored in low temperature to minimize uncontrolled metabolism of yeast. Kefir in low temperature storage of $0-4^{\circ}\text{C}$ may slow the growth of yeast and provide extension in the self-life (Rohmah and Estiasih, 2018). Heat speeds the process for making kefir, so kefir may be produced much more quickly but it may generate over-culture appearance since the number of yeast may go un-controlled growth. Therefore, detecting total yeast of kefir product is important effort to control the quality of this product (Lengkey and Balia, 2014).

Conclusion

Based on this study it could be concluded that total yeast in mango flavored kefir from small industry in Semarang was $1.21 \pm 0.06 \times 10^5$ CFU/ml and in the normal range of standard Codex Stan 243-2003 which requires a minimum total of yeast of 10^4 CFU/ml .

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