Propagation of Kefir in Various Sugar Media

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***Abstract*-- Kefir fermentation in various sugar media were studied, Lactose as a manufacture sugar and Molasses as a natural sugar. The results indicates there was an exponential correlation between fermentation of lactose and the increase of temperature, fermentation was increased at 35°C and decreased at 40°C . also found fermentation Lactose more than Molasses. sugar concentration and media acidity were found as a function of fermentation. The propagation of Kefir in M E M and sugar media also studied and found that in M E M and Lactose more than M E M and Molasses and these more than only M E M , as well as propagation was found in 6th dilution greater than in 5th dilution.**

***Index Term*-- Kefir, Lactose,Molasses ,Sugar,Minimum Essential Media ,Fermentation and Propagation**.

1. INTRODUCTION

Kefir is a viscous, slightly carbonated dairy beverage that contains small quantities of alcohol and, like yoghurt, is believed to have its origins in the Caucasian mountains of the former USSR. It is also manufactured under a variety of names including kephir, kiaphur, kefer, knapon, kepi and kippi , with artisanal production of kefir occurring in countries as widespread as Argentina, Taiwan, Portugal, Turkey and France [1,2,3] .

Kefir grains looks like a waxy cauliflower substance. It proliferates abundantly when given the right environmental conditions **,** Kefir is a group of organisms, mostly varieties of bacteria (Lactobacillus spp., Lactococcus spp., Leuconostoc spp., Enterobacter spp. and Gluconobacter spp.) and several yeasts Saccharomyces spp., Zygosaccharomyces spp., Hahsenaspora spp., Hanseniaspora spp., Honsenizspora spp. and Candida spp[4] **.** Kefir grains they measure 1-3 cm in length, are lobed, irregularly shaped, white to yellow-white in color, a viscous pour able liquid[5]. Slightly foamy body. It is yeasty, acidic, mildly alcoholic, refreshing, slightly effervescent [6] . It is home-produced drink made by adding of kefir grain to sugar solution in water and incubating this mixture at 20-25 °C for at least 12 hours, and then separation of kefir grain to other production [1,4,7,8,9,10]. Grains are kept viable by transferring them daily into fresh milk ,In addition, washing grains in water also reduced viability,Low temperature storage appears to be the best way to maintain kefir grains for long periods [11] .

The major factors affecting rheological properties of kefir are the chemical composition of the milk used for its production, the starter culture, the incubation temperature, the thermal processing of milk .

The yeasts in kefir is less than kefir bacteria, although it is obvious that the yeasts in kefir grains provide an environment for the growth of kefir bacteria, producing metabolites that contribute to the flavour and mouthfeel of kefir [12] . The properties of yeasts found in kefir grains vary. For example, some of the yeasts found in kefir grains are capable of fermenting lactose, while some are not. Also, it has been observed that some types of yeasts are located at the surface of the grain, while others inhabit the interior. It may be that yeasts located at different locations in the kefir grains play different roles in the fermentation process [13] .

yeast is important in kefir fermentation because of the production of ethanol and carbon dioxide. Kefir grains usually contens lactose-fermenting yeast (Kluyveromyces lactis and marxianus, Torula kefir) as well as nonlactose-fermenting yeast (Sacharomyces cerevisiae) [12,14] . The major end products of the fermentation are lactic acid, acetaldehyde, diacetyl, acetoin, ethanol, carbon dioxide and free fatty acids, for [15,16,17] . kefir is not produced yet industrially . However, scientific interest in kefir and the promotion of its industrial production are growing because of its health benefits [18] **.** The objective of the present work is to evaluate the effect of fermentation temperature and consuming of sugar in various sugar media , as well as evaluate the suitable medium to propagation kefir**.**

2. EXPERIMENTAL

2.1 kefir Activation

The grains were grown for 1 week at 28 °C , Kefir grains was transferred into a clean baker then adding milk and gently stir with a soft edged Plastic spoon, so as not to break up the Kefir grains. Cover the baker with muslin .1 Cup of milk per 2 heaped tablespoons of Kefir , Store the culture in light out of direct sunlight on a bench, for about 24 hours, giving it a gentle stir or shake up to two or three times during that period. Once the Kefir has cultured to your liking, strain it through a plastic sieve used to separate the curd from the grains. Pour the curd back into a container and refrigerate. Rinse the baker and put the Kefir grains after washed with sterile water into the clean baker with fresh milk, The medium was changed daily [19] .

Kefir grains

2.2 Preparation of media kefir

Kefir fermentation in two different media, including molasses and lactose, the first media (molasses) was enriched with 10 % molasses , placed in a baker 100 ml of media with 250 g kefir the glass baker covered with muslin , Store the culture in a light out of direct sunlight , for about 24 hours and incubated at 25 °C, stir two or three times during that period. then strain it by using a Plastic sieve to separate the curd from the grains and Retains suspension In the fridge to measure Acidity and sugar concentration , used the same method for incubation at 30, 35 and 40°C . The second media lactose used the same steps to prepare molasses[20] .

2.3 Propagation of kefir

Kefir fermentation in three different media , including Molasses and Minimum Essential Media ,Lactose and Minimum Essential Media and Minimum Essential Media (MEM)only. Prepared 1.665 % of MEM ,10 % Molasses and 10 % Lactose , placed in the first baker (80 ml MEM +20 ml Lactose +25 g kefir), the second baker (80 ml MEM +20 ml Molasses +25 g kefir) and the third (100 ml MEM+25 g kefir) Covered each of bakers with muslin, Store the culture in a light out of direct sunlight , for about 24 hours the incubation at 25 °C, Stir two or three times during that period. then strain it by using a plastic sieve to separate the curd from the grains and retains suspension In the refrigerator to measure acidity and absorbance and colonies count .

2.4 Enumeration of yeasts colonies

A serial dilution was prepared by mixing 9 ml of sterile saline solution with 1 ml of the grains suspension (Molasses + MEM , Lactose + MEM And MEM only) .then filtrate 10 ml from dilutions 5^{th} and 6^{th} by millipore filter 0.22 , transport of the filter paper on Sabouraud Dextrose Agar plates , after cooling the agar to 50°C . The plates were incubated at 28 °C for 3 days in an anaerobic atmosphere **.** After incubation the viable colonies count was enumerated on a proper serial dilution [21] .

2.5 Estimation of sugar concentration by anthrone method

Anthrone reagent was prepared by dissolving 0.2 g of anthrone in 100 ml of concentrated sulfuric acid , sugar samples were prepared by filtration of Kefir water after fermentation using Whatman filter papers. adding carefully on the test tube wall 4 mL of anthrone reagent to one mL of each Kefir filtrate water , The test tubes placed with water bath (80°C) for 10 min ,covered the test tubes with a sheet of marble to prevent Evaporation of water then cooled the test tubes to the room temperature then measuring the Absorbance with Spectrophotometer at 620_{nm} [22].

2.6 Estimation of acidity

Using neutralization method (acid base titration) to calculate the amount of acid produced through Kefir fermentation. The process accrued by transferring 5 mL of each Kefir filtrate water into a conical flask, and titrated against 0.1 M $NaHCO_3$ as a standard basic solution using phenolphthalein as indicator, to calculate the volume (n.of mL) of base equivalence to 5 mL of acidic solution (Kefir filtrate water)[23] .

3. RESULTS AND DISCUSSION

3.1 Effect of temperature on Kefir fermentation

Kefir fermentation in presence of sugars at different temperature was found producing acid through fermentation. Acidity of fermentation solution increased as temperature increased as shown in (figures 1,2) an till 35° C which was in a maximum, while fail at 40°C. These results indicate that acidity as a function of fermentation. Similar results were reported for yogurt samples incubated at 37°C ,40°C and 46°C [24], and also Similar results were reported for bovine milk samples incubated at 20°C,25°C and30°C , According to that, the increase in the incubation temperature resulted in increased Acidity values can be attributed to the activity increase of the bacteria and yeasts [20] .

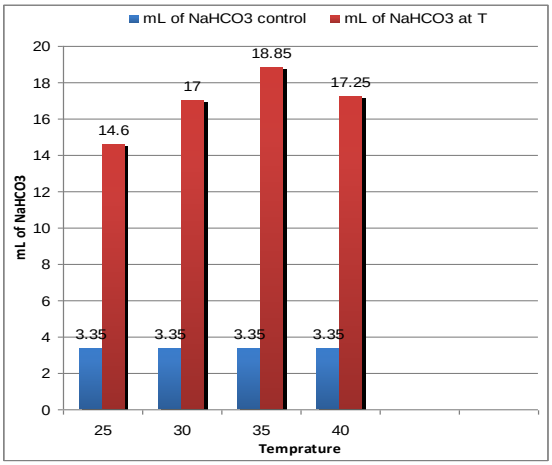

Fig. 1. Acidity (mL of 0.1 M $NaHCO_3$) of Molasses medium increased through fermentation of Kefir at different temperatures .

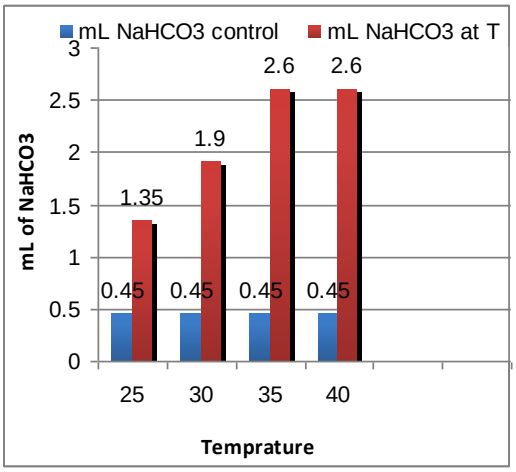

Fig. 2. Acidity (mL of 0.1 M $NaHCO_3$) of Lactose medium increased through fermentation of Kefir at different temperatures .

Different incubation temperatures resulted to a varying absorbance in kefir fermentation. (figure 3,4)showing high consuming of sugar with absorbance of at 35°C, but less consuming of sugar at 25°C.These results of sugar consuming indicate that sugar concentration as a function of fermentation.

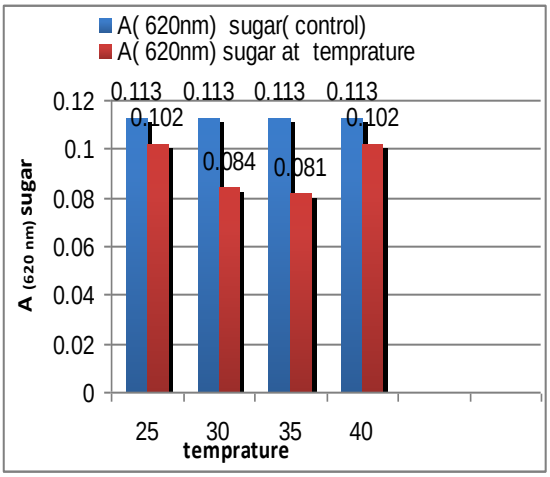

Fig. 3. Molasses concentration decreased through fermentation of Kefir at different temperatures.

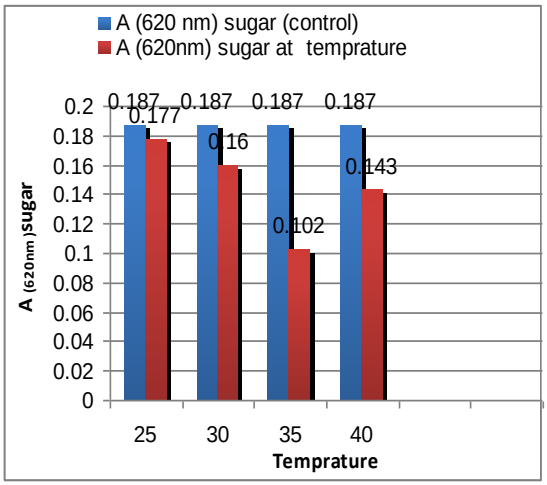

Fig. 4. Lactose concentration decreased through fermentation of Kefir at different temperatures.

3.2 Change of acidity and consuming sugar through propagation

Change of acidity and consuming sugar according to the used medium for propagation of Kefir at 25°C .(Figure -5) appearing increase the Acidity in (Lactose + MEM) was 4.4 mL of 0.1 M $NaHCO_3$ but in (Molasses + MEM) was 4.2 mL of 0.1 M $NaHCO_3$,Whereas decreased in (MEM) to 1.3 mL of 0.1 M $NaHCO_3$.(Figure -6) showing high consuming of sugar with absorbance in MEM + Lactose then MEM +Molasses ,but less consuming of sugar in MEM only. These results indicate to the best medium for propagation kefir it Lactose + MEM.

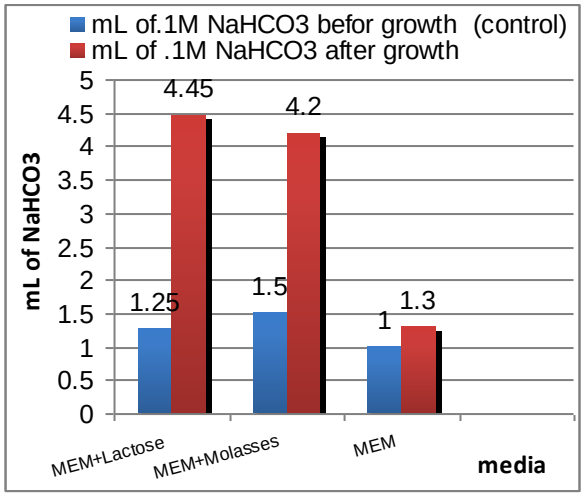

Fig. 5. Changes of acidity through propagation of Kefir at 25 °C .

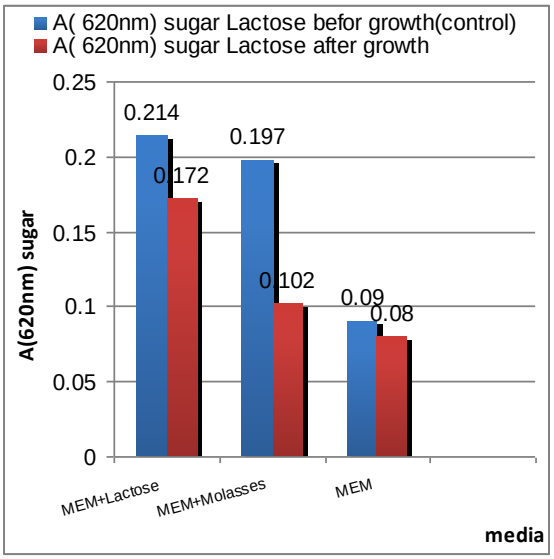

Fig. 6. sugar concentration decrease through propagation of Kefir at 25 ˚C .

3.3 Enumeration of yeasts colonies

The viable cell numbers of the yeast in three media of kefir studied on SDA ,the cell count in 5th dilution show $10.5*10^6$ in Lactose + MEM , $2.5*10^6$ in Molasses + MEM and $2.1*10^6$ in MEM only (Figure -7), whereas the cell count in 6th dilution show a difference $84*10^6$ in Lactose + MEM , $80*10^6$ in Molasses + MEM and $37*10^6$ in MEM only (Figure -8) .These results indicate that Kefire propagation in a maximum when accrued in MEM+ Lactose media and upper dilution (6th dilution), because consuming sugar, specially Lactose by Kefir through propagation, and dilution allowed to free propagation (not crowded).

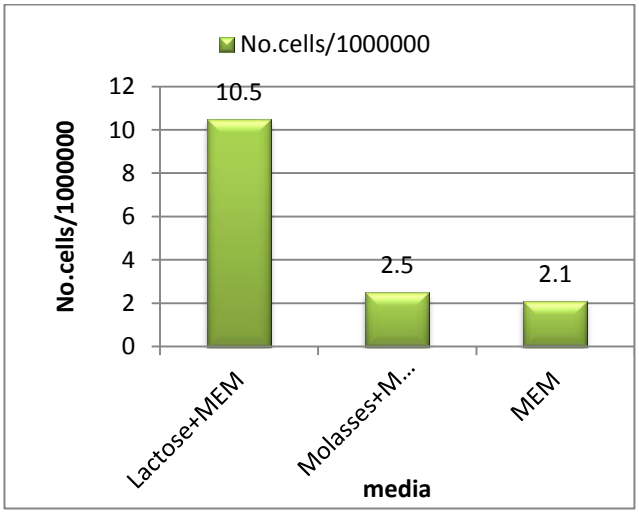

Fig. 7. number of cells through Kefir of propagate in different media at25 °C in 5th dilution.

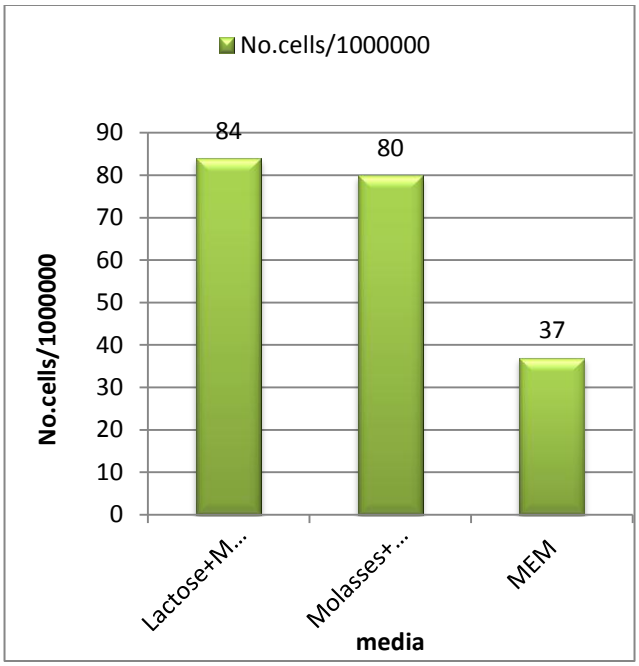

Fig. 8. number of cells through Kefir of propagate in different media at25 °C in 6th dilution.

4. CONCLUSION

This study is important in understanding the mechanism of kefir grain formation and growth because it explores the relationship between fermentation media and the kefir microorganisms . The production of kefir depends on the synergistic interaction of the microflora in kefir grains. During the fermentation process, the yeasts and bacteria in kefir grains produce a variety of ingredients that give kefir its unique taste and texture. After fermentation, the finished kefir product contains many ingredients that are proving to be bioactive. in the present study,Kefir samples at 35°C showed increased acidity while at 25°C exhibited reduced acidity, also Kefir samples at 35°C showed the highest values of consuming sugar, while at 30°C exhibited the lowest consuming sugar when fermentation kefir in Molasses and Lactose.

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