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# PRODUCTION OF PROBIOTIC YOGHURT BY *ZIZIPHORA CLINOPODIOIDES* ESSENTIALOILS, AND CHEKING THE VIABLITY EFFECT OF THAT UPON *LACTOBACILLUS ACIDOPHILUS* AND *BIFIDOBACTERIM BIFIDUM.*

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**ABSTRACT**

In recent years, the probiotic bacteria, as the food additives, have been introduced into numerous foods, of which the dairy products especially yoghurt has played an important role in carrying these bacteria (such as *B. bifidum* and *L. acidophilus*). The purpose of this study, determine the effects of different *ziziphora Clinopodioides* (50, 100 and 150 micrograms), in two passages after and before incubation, on the growth of two probiotic bacteria (*Bifidobacterium bifidum* and *Lactobacillus acidophilus*) in yoghurt produced. The *ziziphora Clinopodioides* yoghurt was produced by Tamime (standard methods). The products were then examined in terms of sensory method, pH, acidity and microbe counting during the incubator setting period and their respective permanence. The results in statistical–descriptive test were analyzed using SPSS software version 16 system. It was repeated three times, respectively. The sample containing 50 microgram of thyme essential oils had the most survival of probiotic bacteria at the first day of storage and the least survival of probiotic bacteria after 28 days of storage is related to control sample. In the case of essentialoils addition stage (before or after inoculation with probiotic culture), addition of essentialoils before probiotic culture increased the survival of bacteria. Between the added bacteria, the survival of *Lactobacillus acidophilus* or *Bifidobacterium bifidum* used separately was higher than the combined samples. The count of Bifidobacterium did not decrease lower than $10^7$ CFU/ ml during storage time and the count of *Lactobacillus acidophilus* during 21 days of storage was $10^7$ CFU/ml. the least decrease of pH and increase of acidity was related to sample with 50 microgram of thyme essential oils which's essential oils was added before inoculation with probiotics and in probiotic samples containing both the bacteria and the most decrease of pH and increase of acidity was seen in control samples. The sample with 50 microgram of thyme essential oils which was inoculated with just lactobacillus before adding essential oils had the most syneresis during 28 days of storage at 4 ˚C and control sample had the least syneresis at the same conditions. In general, addition of 50 microgram of thyme essential oils before inoculating with combined probiotic culture was determined as the best treatment.

**KEY WORDS:** Yoghurt, probiotic, *Ziziphora Clinopodioides, Lactobacillus acidophilus, Bifidobacterim bifidum*

  

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## INTRODUCTION

Yogurt is a fermented dairy product which is popular around the world. In recent years, by the use of probiotic bacteria, a product named probiotic yoghurt has been produced which is known as a healthy and beneficial food. Flavoring compounds are added to yoghurt formulation to improved survival and activity of probiotic bacteria and to maintain the technological features of the probiotic yoghurt (Mortazavian & Sohrabvandi, 2006). In recent years, the probiotic bacteria, as the food additives, have been introduced into numerous foods, of which the dairy products especially yoghurt has played an important role in carrying these bacteria (such as ***Bifidobacterium bifidum*** and ***lactobacillus acidophilus***). Probiotics are recognized for their applications in dairy products, particularly yoghurts and the market for these products is still rising. To achieve the health benefits of probiotics should be their number to $10^6$ log CFU/g. This is a standard for the production and sale of products whose names they are given probiotics (Lourens Hattingh *et al*., 2001). However, in commercial products various probiotic ***lactobacillus*** and ***Bifidobacterium*** show a decline in their viability during product's shelf life (Medina *et al*., 1994; Hull *et al*., 1984). Recently biotechnology has led to a great improvement in the food industry. And food diversity is increasing. Among these, probiotic yoghurt, are known as a suitable environment for probiotics growth. Also, according to the therapeutic effect, this product has been popular among people (Guler-Akin *et al*., 2007). Probiotic organisms especially ***Bifidobacterium*** grow slowly in milk due, in part, to their lack of proteolytic activity, thus requiring the incorporation of essential growth factors such as peptides and amino acids to enhance their growth (Akalin *et al*., 2007). Considerable studies have been conducted to growth and survival improved of probiotics in yoghurt fermentation was conducted as vitamin enriched protein hydrolysate, Amino nitrogen and whey protein concentrate (Amatayakul *et al*., 2006; Joo *et al*., 2001) to enhance the flavor as well as the nutritional quality (Shori *et al*., 2011). Recently, the design and production of plant-based probiotic products have received much attention chiefly due to their natural health benefits (protein, fiber, vitamin and salts) and also because of the variety in their production (Lopez-Malo *et al*., 1995). Therefore, it seems that the issue of producing probiotic foods with appropriate qualities will be a major research topic for prospective researchers. Sensory properties rather than medicinal effects play the most important role in their daily consumptions. Among the fermented probiotic products, the probiotic yoghurt is popular worldwide for its unique sensory properties (Mortazavian *et al*., 2006; Medina *et al*., 1994). Cacooti, scientific name, Tenuir Ziziphora, is of Mint families. The essence of this plant is achieved by leaves distillation and contains some compounds such as Timol, Carvacrone, Bourneal, Linalool, and Bournil. (Rejhan, 1379). Also, it has some antimicrobial and anti-fungal affects and is used in anti-cough drops and expectorant syrup. Also stimulates food consumption, increasing digestive enzymes excretion, increasing digestion and absorption of nutrients, and immune system agitation are of the other properties of the plant (Heidari-1390). Also, in this research it has been assumed that adding Cacooti essence in probiotic yoghurt causes growing, more probiotics settling and the products tissue. The purpose of this study, determine the effects of different doses of *ziziphora Clinopodioides* essential oils, in two passages After and Before incubation, on the growth of two probiotic bacteria (***Bifidobacterium bifidum*** and *Lactobacillus acidophilus*) in yoghurt produced. Mr. Sangeatash and the colleagues, 2005, reviewed the effect of wild Cacooti on starter yoghurt bacteria. So, they provided different yoghurt samples with different wild Cacooti essence with four thicknesses (0, 1000, 2000 and 4000 mgr/li). The results showed significant decrease of starter bacteria during protecting. It was cleared that Cacooti essence didn't have any effect on the

  

living of yoghurt starter bacteria, but 4000 mgr/li density of Cacooti essence from 17th day had a significant decrease of the living of yoghurt starter bacteria. Mr. Shafei and the colleagues (1999), studied anti-microbial effects of Cacooti essence compound on the yeast of *Keloviromices markcianous*, and they reported that the least prevention percent of Cacooti essence in environment was 25%.

## MATERIALS AND METHODS

### Materials

Materials included Cacooti essential oils, Low-fat sterilized milk (1.5% fat content). Probiotic lyophilized packages of *Lactobacillus acidophilus* nu-trish® LA-5 and *Bifidobacterium bifidum* nu-trish® BB-12 were prepared from (CHR Hansen Company, Denmark)

### Essence preparing

In order to prepare essence, first dried Cacooti is grinded and then or essential oil extracted with water by the way of distillation in kelonjer machine for 5 hours.

### Sample Preparation

In order to produce the of probiotic milk containing probiotic bacterium *B. bifidum*, *L. acidophilus*, and mix them, four containers, containing 1 L of low-fat milk were Labeling as four groups. $1.7\times10^{12}$ CFU.ml$^{-1}$ starter was added directly to all the containers, for *L. acidophilus* milk, $4.9\times10^{12}$ CFU.ml$^{-1}$ for *B. bifidum* milk and $3.3\times10^{12}$ CFU.ml$^{-1}$ for mix them. This was incubated at 38 ° C and acidity measurements were performed at different times until reaching 42°Dornic (Standard and industrial search of Iran). The samples were then taken out of incubator and transferred to a refrigerator and stored at 2°C. This process done for each of the starters, separately and mix them.

### Preparation of Before Passage

The second passage, in order to produce probiotic yoghurt, 4 containers were provided and 1 liter of low-fat milk and 15 ml of probiotic milk from the first passage and various concentrations Cacooti, (0, 50, 100, 150 mg/L) were added respectively to the containers and mixed properly, all samples were incubated at 38 ° C (Standard and industrial search of Iran). And acidity measurements were performed at different times until reaching 72°Dornic. Finally, the samples were placed in a refrigerator at 2 ° C.

### Preparation of After Passage

The purpose of After incubation sample, Add to vanillin has stirred probiotic yoghurt. In this passage and various concentrations cacooti (0, 50, 100, 150 mg/L) were added respectively to the containers containing stirred probiotic yoghurt, from the second passage control samples.

Having produced the above-mentioned products, all products were stored in a refrigerator for 28 days. During this period, each sample was tested for acidity, pH, and sensory properties and microbial counts after 1, 7, 14, 21 and 28 days. In this study, bacterial counts were performed by the direct method. Also after 10 days sampels were evaluated for sensory properties, (aroma, scent, color, thickness and taste) the questionnaire by 30 peoples. The respondents were asked to rate the

  

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factors on a scale ranging from very good, good, medium, weak and very weak. The results were analyzed in a statistical descriptive test by SPSS version 16 software.

**Statistical Analysis**

All the above products and experiments were repeated three times. And for data analysis SPSS software was used.

## RESULTS AND DISCUSSION

### pH and Acidity examination

Acidity and pH examination during 28 days showed that in all of samples decreases by passing the time, that pH decrease and acidity increase in yoghurt is because of organic acid producing, growing simulation and probiotic bacteria activity. This is while that increasing the concentrations of Cacooti essence in some samples causes acidity decrease and pH increasing. Examining these results showed that there is basic statistical difference among different samples of yoghurt in 5% level. Control sample comparison other samples, had less pH decreasing.Vossoughi and collegues (1388), in a research examined the effect of Cacooti as current flavours in producing economical churned sue milk, on the ability of two types of *Lactobacillus acidophilus* & *Bifidobacterium bifidum* survival. On this base during keeping some *Bifidobacterium bifidum* to the number of primer ones, declined on average 2/5 Log. This is while that some *Lactobacillus acidophilus* reached to 0 in the 18$^{th}$ week. Also, pH & acidity changed slighty during conserving. The results of statistical analyzing showed that there is meaningful difference taste among all types of churned sour milk. (P< 0/05). Also, the average comparisons showed that the churned sour milks containing Cacooti water got a higher scor tastely rather to normal one. Also, previous passage samples contained more pH rather than next passage, the reason of which, microbicide of Cacooti essence and it's effect on the growing environment on the starter and probiotic bacteries can be mentioned, that decrease acidite producing, neaverly. These results hadn't contradicted with Cailasapati and Ribecca studies in (1997). They supposed that probiotic should be added to the food compound before or with the rennet, otherwise not growing will be observed. It shows the importance of high accelareting probiotic growing in the primary stage of green-house putting.

Among probiotic bacteries, samples containing two bacteries mixing, *Bifidobacterium bifidum* and *Lactobacillus acidophilus* witnessed lower pH, sequentially. In previous samples containing *Lactobacillus acidophilus* a significant difference was seen among pH with increasing essence (0-150 mg/L) thickness during 0 to 28 days. (p < 0/5)

3-2- Probiotics growing and their living

As table (3-1, 3-2) shows, using essence in 50 and 100 mgr causes increasing probiotic bacteria living, while that in 150 thickness it has had negative effect and has caused decreasing their living-every likely Cacooti antimicrobial characteristic in high thickness has effected on probiotic bacteria. This affect was less on *Bifidobacterium bifidum* and has increased its living. Also, in adding essence difference in %0/05 level was significant, and adding essence before inoculation, increased living, in which it is just able with antimicrobial Cacooti essence and decresing antagonist bacteria decrease and increasing probiotic bacteria growth.

  

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**Table 3-1 probiotic factory's living change procedures in yoghurt samples with essence A.**

| Essence A | 0 | 50 | 100 | 150 | SEM |
|---|---|---|---|---|---|
| Number of L bacteria | 6.989[a] | 7.189[b] | 7.146[b] | 6.988[a] | 0.028 |
| Number of B bacteria | 6.901[a] | 7.623[c] | 7.639[c] | 7.478[b] | 0.028 |
| Number of L bacteria in mixture of L+B | 5.713[a] | 7.189[c] | 7.146[c] | 6.988[b] | 0.028 |
| Number of L bacteria in mixture of L+B | 6.707[a] | 7.623[c] | 7.663[c] | 7.478[b] | 0.028 |

**Table 3-2 probiotic factory's living change procedures in yoghurt samples with essence B.**

| Essence B | 0 | 1 | 2 | SEM |
|---|---|---|---|---|
| Number of L bacteria | 6.989[a] | 7.282[b] | 6.933[a] | 0.028 |
| Number of B bacteria | 6.901[a] | 7.748[c] | 7.412[b] | 0.023 |
| Number of L bacteria in mixture of L+B | 5.713[a] | 7.282[c] | 6.933[b] | 0.023 |
| Number of L bacteria in mixture of L+B | 6.707[a] | 7.748[c] | 7.412[b] | 0.023 |

Adding Cacooti essence to probiotic yoghurt causes more probiotic breeding to the witness sample, significantly. But the number of probiotic bacteria in all of the samples during 28 days keeping decreased, which this decline in the samples containing *Lactobacillus acidophilus* was more than samples containing *Bifidobacterium bifidum*, that confirmed with Daveshah's results in 1997, who had used probiotics in order to keep probiotic's survival.

The most numbers of Lacto after 1 day keeping related to ka sample containing 150mg/l Cacooti essence and after 28 days related to 50 mg/l Cacooti essence, while that probiotic's survival in pre-samples was more. In a study by Boolin and collegues (1998), *Lactobacillus acidophilus* starter's livability and conducted that during 35 days, keeping in cold condition, the number of *Lactobacillus acidophilus* decrease. *Lactobacillus* livability in a sample containing mix of two bacteria was seen less than *Bifidobacterium* (chart 3-1). Beside, in first days of keeping samples containing 100 mg and in the final days, the samples containing 50 mgr. Cacooti essence in pre-samples had highest living affect on *Bifidobacterium* growing. Simsick and collegues (2005), showed the number of *Streptococos termophilous* and *Lactobacillous bolgaricoos* In Iran samples (Turkish) produced with spearmint, garden thyme and garlic and the witnessed sample during keeping time reduces meaningfully, but it's mentioned effect on the starter bacteria in comparison with the witnessed samples are not meaningful.

  

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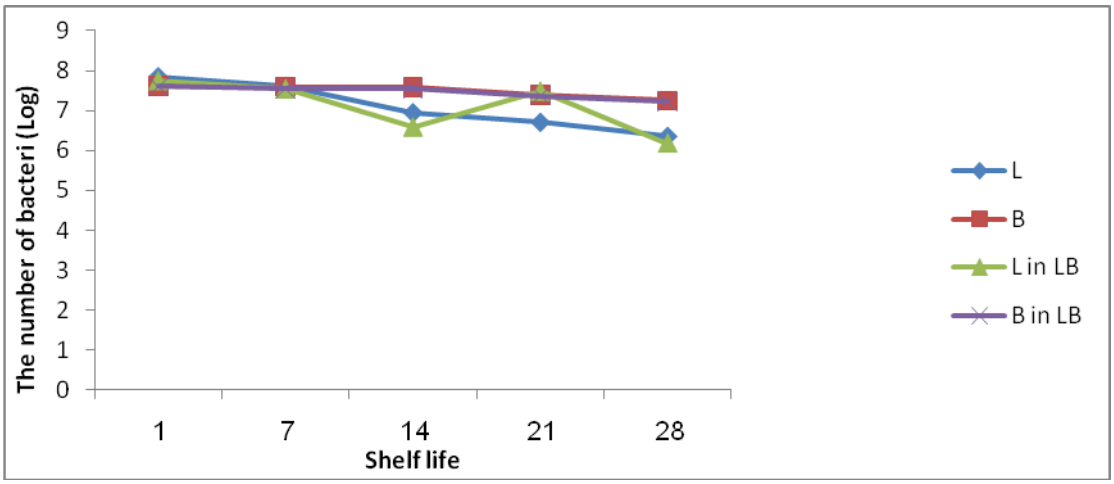


**Chart 3-1- Probiotic bacteries livability producers in probiotic yoghurt Cacooti essence.**

Akalin and and collegues (2004), studied yoghurt bacteria growing and *Bifidobacterium bifidum* in the yoghurt containing fracto aligo saccharid for 28 days in 4 C, and they concluded that the numbers of bactories decrease during keeping period. But the percent of *Fructooligosaccharide* on the *Bifidobacterium bifidum* growth is very effective. Cristo & Collegues in (2003) assessed the effect of milk solid matter, the percent of inoculation and green house putting temperature on the bacterial growing of inoculated milk with *Lactobacillus Paracasei* as well as yoghurt bacteria and they announced that primate milk solid mature has a positive effect on the growing of this bacteria. Also, green-house putting temperature has distinguished effect on growing and acidification, and in the least temperature (36-38 °C), highest growing is observed.

Oost lieh in (2004), announced that among different temperatures, the growing speed of 6 probiotic's types in 37 °C is observed more than the rest.

3-3-Sensory properties and sinersis

According to the present study the percent of water during 28 days keeping and related to the yoghurt samples containing *Lactobacillus* and it was more in the yoghurt containing *Bifidobacterium*. About *Lactobacillus* yoghurt the sample witnessed the least water and in the rest samples, 50 and 100 mg thickness had the least water.

While that the results of probiotic churned sour milk comparison with the witnessed sample in Tehrani and collegues studies in 1384, showed that this bacteria can accelerate the process of decreasing yoghurt bacteria and help to decrease the acidificationafter producing it during keeping. Adding *Lactobacillus acidophilus* hadn't any meaningful difference on the stability of probiotic sample during keeping in comparison with the witnessed one. And percent 2 of phasing on the two samples from first to 17$^{th}$ was severe and after that it was slowed.

  

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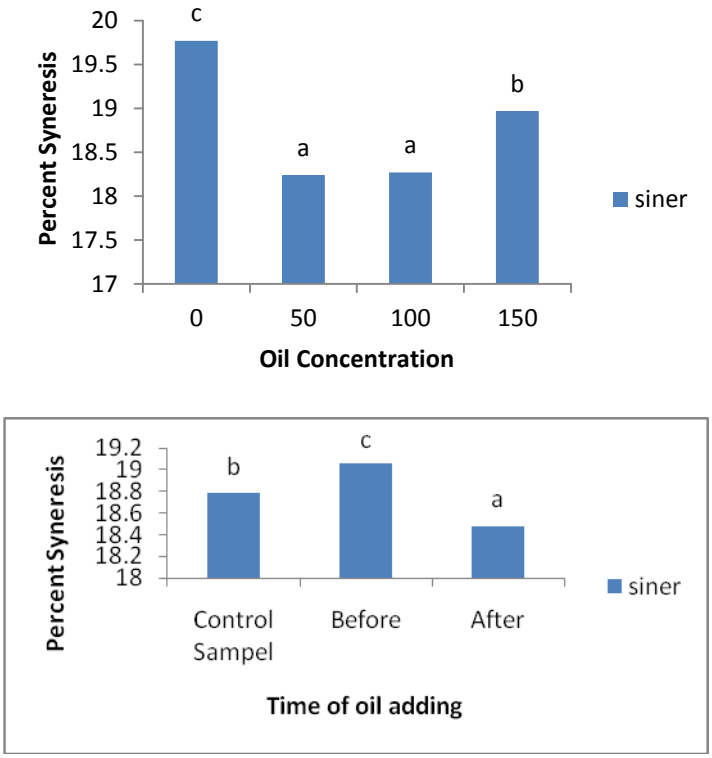


**Chart 3-2- effect of the kind of adding method assence on sinerciss.**

The kind of used bacteria has affected the taste and when both probiotic bacteria were used together, it had a higher point. There was not any significant different among probiotic bacteria about it's odor. But all 3 passage had high advantage rather than witnessed sample. The witness was more in witnessed yoghurt , and at least it didn't show difference ( chart 3-3 & 3-4).

  

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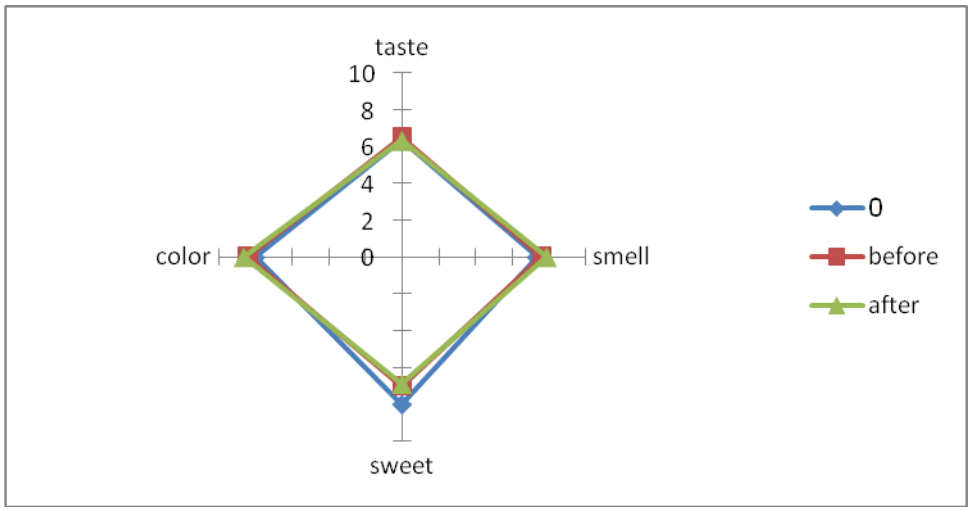


**Cart 3-3-The effect of method type of adding essence on feeling characteristics.**

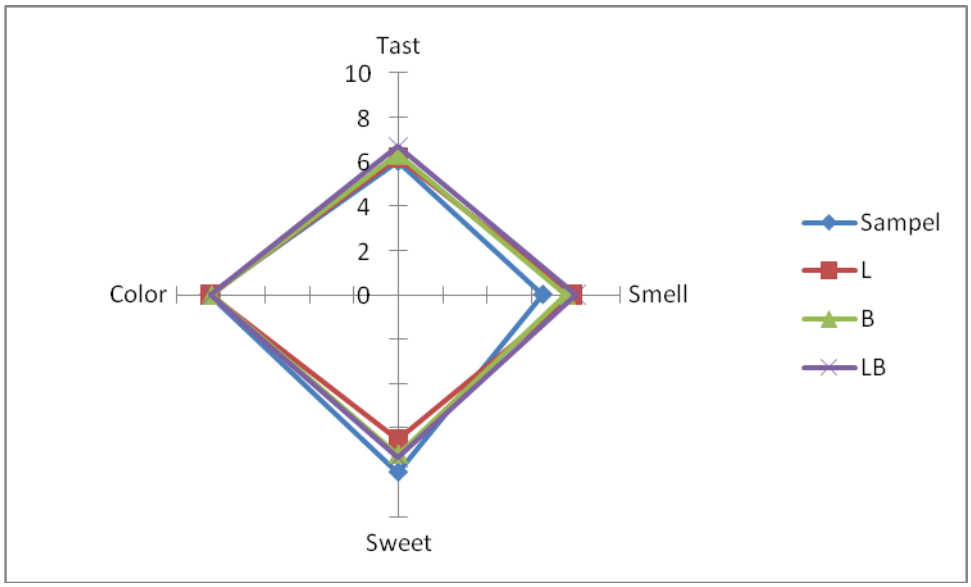


**Chart 3-4- The effect of the kind of bactory on the feeling characteristics.**

## CONCLUSIONS

In order to increase the number of *Bifidobacterium bifidum* and *Lactobacillus acidophilus*, especially *Bifidobacterium bifidum*, Cacooti essence can be used. Adding Cacooti essence about 50 mg had positive effects on decreasing sinersis, pH and acidity. The reason of this problem can be killing other bacteria and microbicide effects of Cacooti essence on un-probiotic bactoria. Acceptability of past passage samples rather to the next passage samples was more. Also, this study showed that adding 50 mg Cacooti essence before inoculation, causes recovery of all of feeling characteristics of this product, including taste, flavor, odor, color, and switness. This is because of positive effect of Cacooti essence and Iranian temperature familiarity with Cacooti taste in other products, such as churned sour milk. The best time for keeping announced 20 days.

  

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