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Effect of grape seed extract (GSE) on the shelf life and microbial load of pasteurized milk in ambient temperature

M.Ahmadian, A.Mohamadi Sani*, A.Arianfar

Department of food science, Quchan Branch, Islamic Azad University, Quchan, (IRAN)

E-mail : msani@iauq.ac.ir

ABSTRACT

The aim of this study was to survey the effect of grape seed extract (GSE) on microbial load reduction and increase in shelf life of pasteurized milk at ambient temperature. For this purpose, different percentages of GSE were added to the milk and the total microbial load test, coliform and acidity tests were performed on at three days. Finally, the statistical analysis performed by Spss-20 software through ANOVA and T-student method. The results showed that, after 24 hours, the milk samples contained 0.5, 1 and 2.5 percent GSE completely were decayed, but milk sample containing 5% of GSE remained up to 72 hour. Statistical analysis showed that, significantly at three days, the total count of microorganisms in the milk sample containing 5% of GSE, increased, but the acidity did not. Accordingly, it was found that GSE at concentration of 5% increased pasteurized milk shelf life at ambient temperature up to 72 hours via inhibition from production of lactic acid by Lactic acid bacteria.

KEYWORDS

Grape seed extract (GSE);
Pasteurized milk;
Shelf life.

INTRODUCTION

Recent research indicates that many healing properties of grapes are undoubtedly related to its seed. Vitamin E, flavonoids, linoleic acid, and compounds called proanthocyanidins are very compact instead of the grape seed. During pasteurization process of raw milk most pathogenic and spoilage bacteria are destroyed. However, this process cannot destroy the thermoduric and soporiferous bacteria[1]. Some of these bacteria, generate extracellular enzymes which are resistant to heat in pasteurization process[2] and eventually intense heat used to solve this problem which causes undesirable changes in sensory and nutritional properties of milk[1, 2]. In a research conducted by Gokturk et al (2004), adding GSE in 0.5 and 1% at mediums of microorganisms, had bacteriostatic effect and had bactericidal properties at concentrations of 2.5 and 5 percent[3]. Kao et al (2010) and baydar et al (2006) showed that using one mg/ml GSE caused 99% inhibition in growth of *Staphylococcus aureus* and also had anti-bacterial effect against many pathogenic and food spoilage bacteria including *Escherichia coli* (*O157:H7*), *S.Typhimurium* and *Salmonella enterica serovar Enteritidis*[4,5]. Rhodes et al, 2006 showed that using GSE (0.25 mg/liter) for 10 minutes decreased the amount of *Listeria monocytogenes* from 10^6-10^7 CFU/ml to zero[6]. In another study by Amir S. et al (2008) GSE was extracted by six solvent systems and the antimicrobial effects of GSE were determined by

MBC[1] method and results showed the high capability of GSE for destroying bacteria[7]. In this study, gram-positive bacteria were more sensitive than gram-negative bacteria. In another study, it was shown that the sensitivity of gram-positive bacteria against GSE was more than gram-negative bacteria[7]. The antibacterial activity of GSE studied by Veronica et al (2011), Results showed that phenolic compounds and catechins in GSE had an antibacterial activity and lethal effect on the gram-positive and negative bacteria such as *Escherichia coli* and *Brevibacterium linens* in dose of 35 (µg/ml) after 13 hours[8]. Minimum inhibitory concentration of GSE for anti-listeria activity was determined as 0.26 (mg/L)[9].

The aim of this study was to determine the effect of GSE on the shelf life of pasteurized milk in ambient temperature without effect on the sensory properties of milk.

MATERIALS AND METHODS:

GSE capsules purchased from Good, N Natural Company, each capsule contained 100 mg of GSE with a purity of 90-95% of compounds named proanthocyanidins. Milk collected from animal husbandry, which were tested for total microbial load, coliform and acidity after pasteurization. Acidity test of milk (according to ISIRI standard-2852) and microbial tests, including total count of microorganisms (according to ISIRI standard-2406) and coliform count (according to ISIRI standard-2406) were conducted. Milk spilled into four 400 ml sterilized containers with lid and GSE added to them with proportions of 0.5, 1, 2.5 and 5% (w/w). Then every 24 hours, acidity, total count and coliform tests performed on samples with three replications. Total count test done in the 10^{-3} dilutions and coliform count test done in dilution of 10^{-1}. Results analyzed by Spss-20 software using one way ANOVA test. Statistical tests done in level of 0.01 and then T student test evaluated between microbial load and acidity at 0.01 level.

RESULTS AND DISCUSSION

After 24 hours, samples of milk containing 0.5, 1.0 and 2.5% of GSE and the control completely decayed but the sample containing 5% GSE remained normal without changing in organically characteristics. The result of microbial and chemical tests is presented in TABLE 1.

TABLE 1 : Effect of GSE in 5% on acidity, microbial load and coliform of pasteurized milk in ambient temperature during 3 days

Replication	Acidity			Microbial load (CFU/ml)			Coliform (CFU/ml)		
	Day one	Day two	Day three	Day one	Day two	Day three	Day one	Day two	Day three
Repetition one	15	15	15	162	217	295	4	8	14
Repetition two	15	15.5	15	162	217	296	5	9	13
Repetition three	15	15	16	161	216	296	5	8	13

Total microbial load data analyzed by ANOVA. The obtained F value was equal to 40837.08, which is much higher than value obtained from Fisher table and the fact is that during three days the total microbial load of milk was increased, so GSE did not have killing properties for bacteria. The F value at 1% level for the number of coliform was equal to 170.3504, which is higher than the number in the Fisher table. The fact is that during three days the number of coliform in milk was increased. The F value at 1% level for acidity was equal to 0.6004. This result indicates that by addition of GSE to pasteurized milk, ýdespite increasing in the number of bacteria in three days, the ýacidity of milk did not significantly increase. GSE ýshowed inhibitory effect on acidity in pasteurized milk. The relation between number of bacteria and milk acidity was investigated by T-student test at 5% GSE and the obtained value was equal to 6.582. This result was higher compared by result of T-student ýtable which means that with ýincreasing in the number of microorganisms, acidity of milk did not increased; whereas in normal state by increasing in number of ýmicroorganisms, the milk acidity increases. Lack of relationship between milk acidity and ýnumber of microorganisms, only can happen when a counterfeit is done in milk ýto hide the milk acidity as like as adding baking soda. So It can be concluded that GSE at 5% concentration, have prevented the metabolic activity of ýmicrobial cells and despite increasing in the number of bacteria, the ýmetabolic activity of microorganisms and production of compounds ýthat play a role in the increasing the milk acidity have been decreased.

Many researches has been conducted on the antimicrobial effects of GSE in laboratory scale and in vitro conditions not in food model. Of course, the growth of

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microorganisms in food is different from the growth in specific culture.

These effective factors are including:

- Milk microflora[10]
  Researches show that different microorganisms in a food, competes together in consumption of nutrients.
- Microbial metabolites[11]
  Productions of metabolic substances produced by a microorganism can inhibit the growth of another metabolite such as lactic acid produced by lactic acid bacteria.
- Milk acidity[12]
  In the past studies; the effect of increasing or decreasing the milk acidity, was not reported to be effective on antimicrobial activity of GSE.
- Consumption of GSE by microorganisms:
- High content of nutritious compounds in milk:
- Ambient temperature[13,14]
  The growth and proliferation of microorganisms in milk is associated with environmental temperature, this will be dealt with legislation that known as Q10.

In this study by addition of GSE to milk at 5% concentration in ambient temperature, the activity of microorganisms was prevented significantly. The effect of the higher percentages of GSE can certainly achieve better results.

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