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ASSIMILATION OF CHOLESTEROL IN MILK BY KEFIR CULTURES

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SUMMARY

Significant variations in the ability to assimilate cholesterol in milk were observed among 6 kefir cultures. The amounts of cholesterol assimilated during 24 h of incubation and 48 h of storage ranged from 10.8 to 5.3 mg/dl of milk or from 84 to 41% of cholesterol in control milk (12.8 mg/dl).

INTRODUCTION

Several researchers have presented evidence for an in vivo cholesterol-lowering effect of fermented milks and their cultures in humans (Harrison and Peat, 1975; Jasper et al., 1984; Gorbach et al., 1988; Rajala et al., 1988), as well as in animals (Rao et al., 1981; Kiyosawa et al., 1984; Kaup, 1988; Gilliland et al., 1985). However, due to some of the controversial results it is not possible to draw any definitive conclusion on a hypocholesterolaemic effect of cultured dairy products.

Some lactic acid bacteria, bifidobacteria and yogurt cultures were tested in vitro experiments on their ability to assimilate cholesterol in MRS broth containing pleuropneumonia-like organism (PPLO) serum fraction as a source of cholesterol. Gilliland and Walker (1990) reported wide variations in cholesterol-assimilating ability, among 13 cultures of Lactobacillus acidophilus. Also, Rašić, et al., (1992) have observed an active assimilation of cholesterol by cultures of Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Bifidobacterium bifidum and Lactobacillus acidophilus.

The starter culture for kefir is obtained by growing kefir grains in milk. The kefir grains are a unique, specific symbiotic formation composed of a mixture of at least six functionally different groups of microorganisms

including mesophilic hemofermentative lactic acid streptococci, termophilic and mesophilic lactobacilli, acetic acid bacteria and yeasts (Koroleva, 1988). Due to this microbial complexity, kefir grains of various origin are characterized by very distinct physicochemical and microbial properties, (Zourari and Anifantakis, 1988; Üçüncü and Ergülü, 1989).

Available observations on the ability to assimilate cholesterol by lactic acid cultures suggest that kefir cultures could possess the same attribute. This study was to determine significant variations in ability to assimilate cholesterol in milk among some kefir cultures.

MATERIALS AND METHODS

Cultures. Six types of kefir grains were used in this study. They originated from Yugoslavia (type A,B,C and P), Hungary (type M) and Caucase (K). The kefir grains were maintained by routine cultivation as described by Koroleva (1988), with the exception that the UHT sterilized whole milk was used. Each type of kefir grains was subcultured twice in experimental milk prior to further use.

Milk. The milk for experiments was prepared by reconstituting instantized whole milk powder in distilled water to 12.5% total solids and 3.5% fat. The reconstituted milk was heat treated at 95^{o}C for 15 min, cooled to 25^{o}C and than inoculated.

Assimilation of cholesterol. Ability to assimilate cholesterol was determined by inoculating 100 ml milk with 6 g kefir grains. The inoculated milk was incubated at 20^{o}C for 24 h. The grains were sieved out of coagulated milk (kefir culture). The kefir culture was cooled and stored at 10^{o}C for 48 h. Analyses were perofmed after incubation and during the storage of culture after 24 and 48 h. Differences in the amount of cholesterol in the uninoculated control and in the spent milk (culture) samples were taken as amounts of cholesterol assimilated.

Analyses. Cholesterol was determined by using the method of Rudel and Morris (1973). The pH of samples was determined by using an Iskra MA5703 glass electrode potentiometer. Acidity was determined by titration of a 10 ml sample with 0.25 M NaOH and phenolphthalein indicator and reported as % lactic acid. Statistical analyses were performed to determine possible significant variation occurred among mean values for different cultures.

RESULTS AND DISCUSSION

All kefir cultures tested have exhibited an ability to assimilate cholesterol in milk during incubation and storage, Table 1. The highest assimilation was during the incubation, but it continued at a lower rate during the storage. The amounts of cholesterol assimilated by cultures during the incubation time ranged from 8.00 to 2.83 mg/dl or from 62.5 to 22.1% of initial cholesterol contents in the control milks. The total amounts of cholesterol assimilated after a further 24 and 48 h of storage ranged from 8.35 to 3.63 and 10.79 to 5.28 mg/dl, respectively. The amounts of cholesterol decreased during the 24 and 48 h of storage ranged from 65.2 to 28.4% and 84.2 to 41.3%, respectively. Significant variations ($P < .05$) existed among the cultures with regard to their ability to assimilate cholesterol from milk during incubation as well as during storage.

Table 1. Assimilation of cholesterol by kefir cultures during incubation at 20°C and subsequent storage at 5°C

Culture	Incubation 24 h			Storage 24 h			Storage 48 h		
	Mean[1]		%[2]	Mean		%	Mean		%
A	8.00	$(3.6)^{a}$	62.5	8.35	$(3.3)^{a}$	65.2	10.79	$(3.5)^{a}$	84.2
M	7.05	$(2.1)^{a}$	55.0	8.93	$(3.3)^{ab}$	69.8	10.10	$(2.3)^{a}$	79.9
C	6.01	$(3.0)^{ab}$	47.0	6.16	$(3.4)^{ac}$	48.1	6.40	$(3.2)^{b}$	50.0
B	4.37	$(1.8)^{bc}$	34.1	5.88	$(2,1)^{c}$	45.9	6.67	$(2.2)^{bc}$	52.1
K	2.83	$(2.1)^{d}$	22.1	3.63	$(2.0)^{d}$	28.4	5.28	$(2.1)^{d}$	41.3

a, b, c, d Means in same column followed different superscript letters differ significantly ($P < .05$)

[1] Each mean (mg/dl) is from 12 cases carried out in four trials; standard deviations are in parentheses.

[2] Amount of cholesterol decreased in percent of the initial cholesterol content of control milk sample (12.80 mg/dl).

Cultures A, M, and C, assimilating over 6 mg/dl were significantly more active than cultures P and K assimilating less than 3.4 mg/dl during incubation. Also, during the period of storage the cultures differed

significantly in their ability to assimilate cholesterol following the same pattern.

By electron microsopy, cholesterol was localized within the surrounding membrane and triglyceride core of milk fat globules (Martin, 1989). It was found to be highly organized within membrane portions and less organized within triglyceride portions of the fat globules. Cholesterol localized in fat globule membrane was found to be accessible to cholesterol oxidase added to homogenized milk and it was degraded up to 50% (Xiansheng 1990). Our results indicate that the cholesterol in milk is accessible to the cholesterol-degrading enzymic system of the kefir cultures. The observed variations in assimilating cholesterol by different kefir cultures may be of interest in selecting desirable types of kefir grains for the manufacture of a low-cholesterol kefir type beverage.

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