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Pharmacodynamic Effect of Growth of *Saccharomy ces cerevisiae* During Lactic Fermentation of Milk.

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Abstract: The application of yeast like *Saccharomyces cerevisiae* to produce fermented milk was known for ages in the history. It is presumed that the primary objective of producing such fermented milk may have been to get some alcoholic drink along with some nutrition of milk. The pharmacodynamics and pharmacokinetics of such products was not known till the findings of *Saccharomyces boulardi* which did have some useful pharmacodynamic value when consumed. These reports of useful pharmacodynamic values were simply attributed to *Saccharomyces cerevisiae* merely by the similarity they have. In this study it has been attempted to point out that whatever may be the similarity between the 2 yeasts, it is harmful to consume milk products either contaminated by *S. cerevisiae* or have been deliberately added to the product, as the yeast produces a very harmful compound like furfural and its 5-methyl derivative.

Key words: Curd, *Saccharomyces*, furfural, furans

INTRODUCTION

The application of lactic fermentation of milk by *Lactococcus* spp. is well known since centuries (Rodrigues *et al*., 1996). Yeasts have been used along with *Lactobacillus* and *Lactococcus* to produce fermented milks like kefir (Lourens and Viljoen , 2001), acidophilus yeast milk (Lang and Lang, 1975) etc. Besides yeasts contributing to flavor and aroma of the finished product, they have been shown to have tremendous pharmacodynamic properties like direct antagonisms, secretery effects and trophic effects, as a result yeasts are considered as well known probiotics along with lactic acid bacteria (Czerucka and Rampal, 2002). One such trophic effects that has been reported by several workers is that, yeasts prevent attachment of many bacterial toxins to the binding sites on the brush borders of the small intestine cells (Pothoulakis *et al*., 1993; Castagliuolo, 1996). However, most of these reports are from the yeast *Saccharomyces boulardi* though there are plenty of reports of *S. cerevisiae* being used in many manufacturing processes, specific useful effects have not been noted, except everybody claims that since there is hardly any differences between the 2 species of *Saccharomyces,* both the species must have the same pharmacodynamic effects. Actually it is not so, *Saccharomyces boulardii* and *Saccharomyces cerevisiae* differ widely in their metabolic activity. In fact there are no reports or claims of *S. cerevisiae* having any harmful pharmacodynamic effects as a contaminant specially when consumed along with a fermented milk product.

Contamination by other anaerobic microorganism like yeast was never given any importance in dairy industries until the harmful effects like interference in cheese formation was noted (Robinson, 2000). It was fairly recently that spoilage of Mozzarella cheese was observed when made from cow's milk and it was established to be due to oxidation of the proteins and fats as cows' milk contains low levels of tocopherols and other antioxidants (Balestrieri *et al*., 2002). Several spoilage yeasts like *Debaromyces, Kluyveromyces, Hansunela* etc have been found and isolated from lactic fermented milk (Welthagen and Viljoen, 1998). On scrutinizing the reports of certain dairy in our country, who have been complaining of persistent yeast contamination, it was observed that well known and commonly found true yeast which was the cause of the problem, was *Saccharomyces cerevisiae* which was isolated by certain dairies and identified. The contaminating yeast was so severe in certain dairies that it was interfering with the stretchability of the curd which was to be further processed into Mozzarella cheese. *Saccharomyces spp* cannot ferment lactose. Therefore, the lactic acid bacteria ferment lactose into glucose and galactose, of which the glucose is converted into lactic acid. The lactic acid being a primary metabolite is often further metabolized by yeasts which create a congenial environment for the growth of other spoilage microorganisms capable of causing damage to the curd resulting in ultimate damage to the product like cheese.

In our country lactic fermentation of milk (known as curd) is also done by virtually every home using the *Lactococcus spp* and this is served along with major meals like lunch or dinner. The milk used for such a product can be cows' or water buffalo's milk. It is often given as a probiotic to patients suffering from gastric disorders especially those who have been on oral antibiotic therapy for a long time. It helps to check diarrhea in many cases, though not in all cases. There are certain small scale dairies who also prepare such curd and sells to those households where it is not prepared. Random Investigations of some of these curd samples did show the presence of *Saccharomyces cerevisiae* in significant numbers (on an average 10^8 CFU/g). Consumers never complained, because the contaminant never altered the palatability of curd.

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MATERIALS AND METHODS

The curd forming culture, which was of different *Lactococcus* spp., was obtained from a supplier (M/s Duke Thompson, Indore, India) who supplies this to many dairies including some of the multinational dairies. The culture was checked for yeast contamination prior to use. It was only after being thoroughly reassured by the results of testing, it was further taken up for curd precipitation.

The milk used for curd making was homogenized and pasteurized milk with a fat content of 6%, solids non fat content of 8.5% and a pH of 6.7. It also did not reduce methylene blue even in 3 hours of incubation. In spite of all these precautions, the milk was sterilized at 121˚C for 15 minutes in sterile flasks. The culture added to 100 ml of this milk was as per the instruction of the manufacturer. The incubation was carried out at 30˚C for 12 hours. Obviously the curd obtained was a soft curd which was having a even velvety texture with a good smell and a pH of 4.2. Later on this curd was similarly checked for yeast contamination.

Similarly another set of curd was prepared but here yeast: *Saccharomyces cerevisiae* ATCC 3455 was used. This was found to have a very short generation time. The quality of the curd obtained was the same as that which was obtained without yeast. It was checked for number of yeast cells present per gram of curd.During the entire course of this study the medium used to check yeast growth, contamination and also for enumeration, had the following composition: yeast extract 0.4%; malt extract 1% and glucose 0.4%. It was solidified with 2.5% agar-agar. The pH of the medium was 5.5 to minimize bacterial growth. The medium was sterilized at 121˚C for 15 min.

The reducing sugar level in the milk as well as in the curd was checked by the Dinitro Salicylic Acid method (Lindsay 1973) and also the alcohol content was checked both in the milk as well as in the curd by potassium dichromate method (Caputi *et al.,* 1968). The results are recorded in Table 1. The curd obtained by both the methods stated above, was thoroughly agitated and the whey was separated by filtration. The whey was air dried in an oven at 60˚C. It was then solubilized in methanol. The amount of methanol used was the same as that of loss of weight in drying. These solutions were used first for checking different absorption maxima in the UV range in a spectroscopy. The results are as shown in Fig. 1 and 2. The same solutions were then checked by GCMS. The results are recorded in Fig. 3 and 4.

RESULTS AND DISCUSSION

The curd with yeast showed slight stormy fermentation as compared to that prepared without yeast It can be seen from Table 1 that considerable amount of alcohol was produced. This revealed that the yeasts were first producing significant amount of carbon dioxide to reduce the oxygen tension in the milk which could support its growth, beside being provided with the necessary amount of reducing sugar by the *Lactococcus* species growing on lactose. It must be remembered that *Saccharomyces cerevisiae* can not metabolize lactose.The initial pH of the milk was 6.6 & it dropped to 4.2 after 6 hrs. and it did not change even after 10 hrs. in both the studies. This could be attributed to the fact that *Saccharomyces cerevisiae* is not a proteolytic yeast.

It can be seen from Fig. 1 and 3, that propylene glycol, which has an absorption maxima at 230nm (Sinjewel *et al.*, 2007), was being formed by the *Lactococcus spp.* It has antimicrobial activity because of which other contaminants cannot grow in the curd & hence it ensures uniform results batch after batch giving consistent production of curd with same acidity, texture and flavor.

Table 1: Results of curd fermentation in presence of yeast and in absence of yeast

Sr. No	Description	Initial value	Final Value	
			With yeast	With out yeast
1	pH	6.6	4.2	4.2
2	Reducing sugar content	0.23%	0.1%	0.5%
3	Alcohol generated	0.12%	6.1%	Not detected

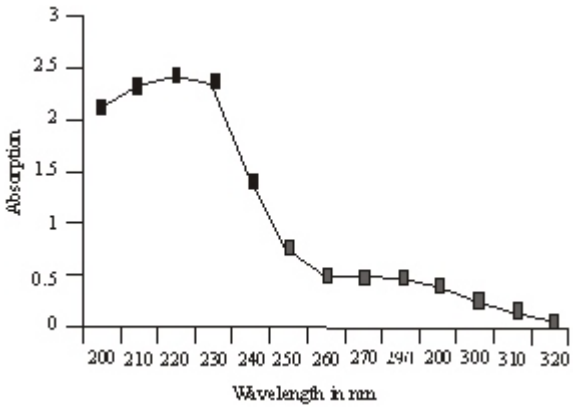

Fig. 1: UV spectroscopy of methanolic extract of the whey during curd formation without the yeast

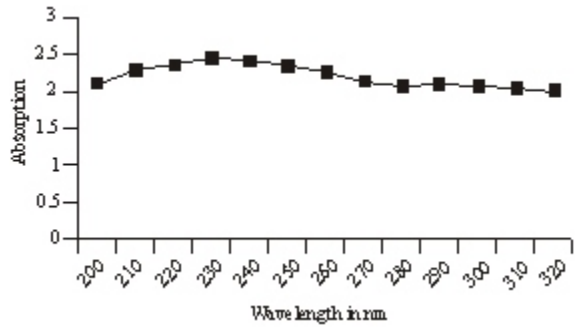

Fig. 2: UV spectroscopy of methanolic extract of the whey during curd formation with the yeast

The GCMS studies of the test & the control were also carried out. The results of the same are shown below:

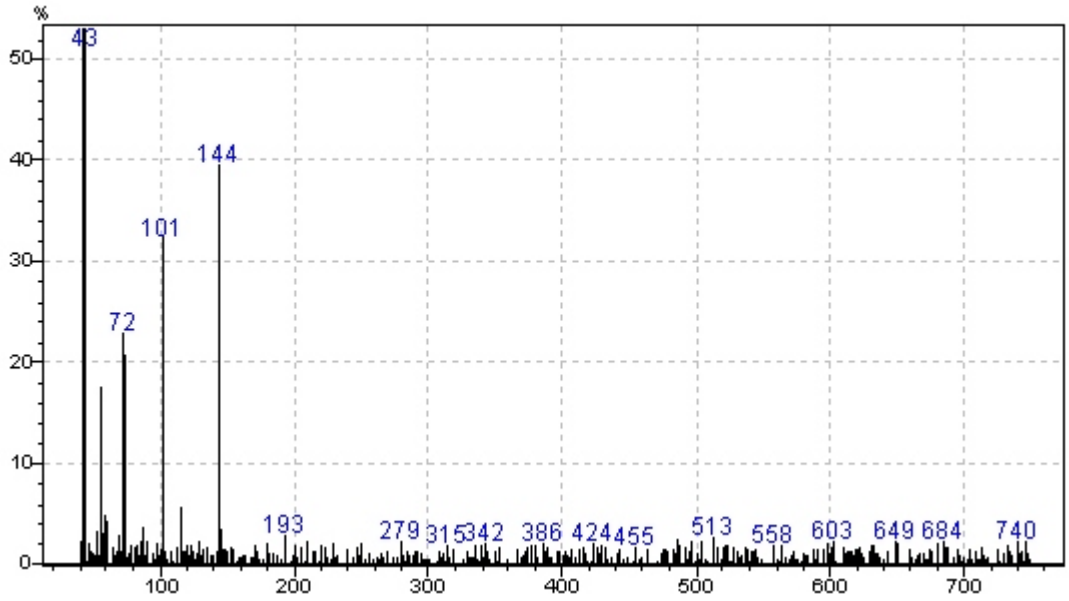

Fig.3: GCMS of the methanolic extract of the whey when curd was prepared without the yeast

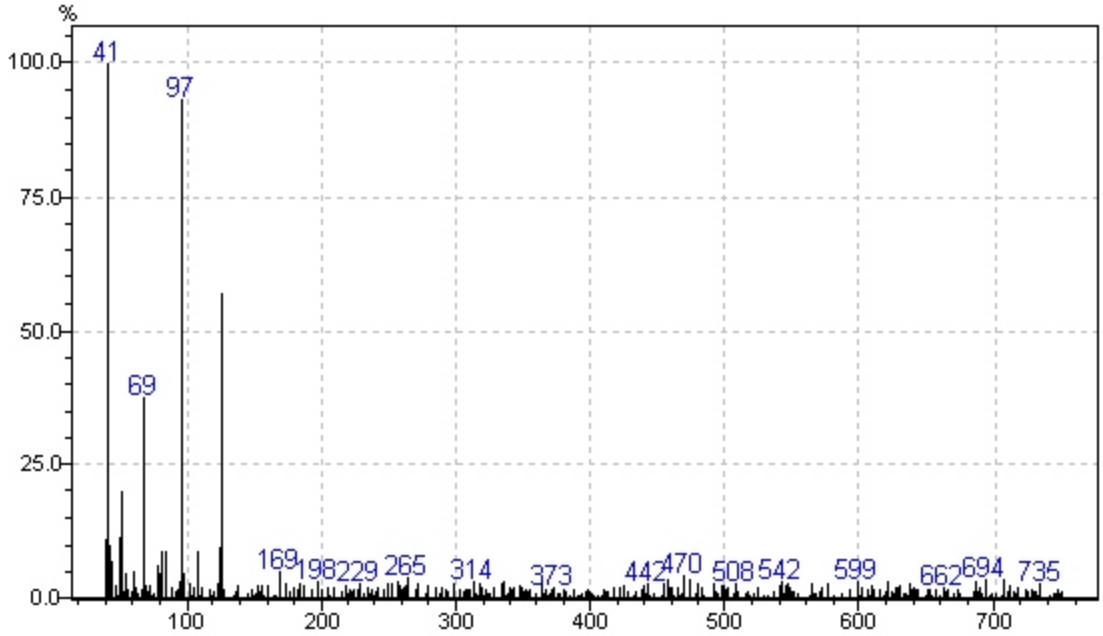

Fig.4: GCMS of the methanolic extract of the whey when curd was prepared with the yeast

However, on examination of figures 2 and 4 it appears that propylene glycol is being removed and a new substance, furancarboxaldehyde and 5-methyl furancarboxaldehyde, which have an absorption maxima at 254-280 nm (Faramarzpour, 2009) and are not antimicrobial, were being formed. The curd was now vulnerable to microbial attack as evidenced by the luxuriant growth of the yeast *Saccharomyces cerevisiae* producing alcohol.

CONCLUSION

It can therefore, be concluded that yeast must be producing the 2 furans as mentioned. Regardless of the mechanisms of the production of these furfurals by *Saccharomyces cerevisiae,* the most important fact is that continuous consumption of furfurals can lead to diarrhea in children and in the infirm as these compounds tend to attach to the brush borders of the intestinal epithelial cells.

The second pharmacodynamics of furfural is its accumulation in the liver leading to hepatic oncogenesis, which ultimately affect the B-cells of pancreas leading to disease like diabetes. Therefore, curds containing the furfurals can not be safe for consumption nor it can be used as a source of probiotics.

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