World Applied Sciences Journal 12 (5): 613-618, 2011
ISSN 1818-4952

Optimization of Kefir Grains Production by Using Taguchi Technique and Mini-Fermentation

Neda Habibi, Sabihe Soleimanian-Zad and Mahmoud Sheikh Zeinoddin

Department of Biotechnology Laboratory,
Food Science Isfahan University of Technology, Isfahan, 84156-83111, I.R. Iran

Abstract: Taquchi design and Mini-fermentation system was used to optimize the kefir grain production. The effects of different parameters including percentage of fat in milk, skim milk proportion, incubation temperature, incubation time, tryptose, glucose, CO_2 concentration and amount of milk on the percentage yield of kefir grain were investigated. Skim milk and low fat milk were the best medium for kefir grain production. Results of Taguchi design L-18 showed that the effects of percentage and volume of skim milk were 46 and 24%, respectively. Effect of temperature (37°C) was only 5.9% and although fat inhibits kefir grain production but its effect is only 1.8%. It was concluded that the Taguchi design is a promising approach to analysis effect of multi variables simultaneously and economically.

Key words: Kefir · Taguchi · Optimization · Mini-fermentation · Grain Production

INTRODUCTION

Kefir is a fermented milk drink produced by the actions of bacteria and yeast contained in kefir grain and is reported to have unique taste and properties. Kefir grains are used as the natural starter in kefir manufacturing and consist of a variety of microorganisms entrapped in a water-insoluble carbohydrate matrix which makes them extremely tough and resilient [1]. The grains are initially very small but increase in size during fermentation but they can only grow from pre-existing grains [2]. Modern kefir grain production is based on continuous cultivation in milk, resulting in biomass increases of 5–7% per day [3]. These grains consist of a complex set of lactic acid bacteria (*Lactobacillus casei, Lactobacillus hilgardii, Lactobacillus delbrueckii spp bulbaricus, Lactobacillus plantarum, Lactobacillus kefir, Leuconostoc mesenteroides* sp *dextranicum and Streptococcus lactis*), yeasts *(Zygosaccharomyces cerevisiae, Torulospora pretoriensis, Candida lambica and Candida valida*) and acetic bacteria embedded in a specific polysaccharide matrix [4]. Peptides and exopolysaccharide are formed during fermentation that has been shown to have bioactive properties such as anti-carcinogenic, anti-mutagenic, anti-viral and anti-fungal properties. Kefir is also a suitable drink for lactose intolerance people. Kefir grains are key ingredient in kefir production and it has been found that the finished product has a different microbiological profile from the grain and therefore can not be used to inoculate a new batch of milk. The complex microbiological composition of kefir grains explains why it is difficult to obtain starter with the optimal and constant composition necessary for a regular kefir production of standard quality [5]. Different studies have been undertaken to establish cultivation conditions such as, grain: milk ratio, cultivation temperature, period of time and conditions prior to separation of grains from the fermented milk, shaking conditions for agitation of milk with the grains through the fermentation process, washing of kefir grains and so on [6]. All these factors influence the microflora of the kefir starter and fermented milk. There are no rules about household manufacture of kefir. Different reports indicate a wide range of grain: milk ratios for kefir making. A critical control point in kefir manufacture to obtain a product with constant quality is the standardization of the kefir grain: milk ratio. Gracielal *et al*. [7] claimed that it is better to use kefir grains as starter for kefir production and, at the same time, to increase the amount of inoculums. The purpose of this study was to investigate the effect of different culturing conditions on Kefir grain biomass increase and to assess more kefir grain production in different media with different ratio by Taguchi design.

Corresponding Author: Sabihe Soleimania-Zad, Department of Biotechnology Laboratory, Food Science, Isfahan University of Technology, Isfahan, 84156-83111, I.R. Iran.
Tel: +98-0311-3913392 & +98-0311-3913392, E-mail: soleiman@cc.iut.ac.ir.

Table 1: Variable and their levels tested by Taguchi design

Factors	Levels		
Fat	0	1%	
Glucose	0.01%	0.25%	0.5%
Skim milk proportion	2%	5%	10%
Temperature	25 ºC	30ºC	37 ºC
Time	20 hr	25 hr	36 hr
Tryptose	0.1%	0.2%	0.3%
CO_2	0.5%	5%	10%
Skim milk (vol)	0.5 ml	1 ml	1.5 ml

Table 2: Taguchi experiment design L-18

	Factors and Levels							
Experiments	Fat	Glucose	Skim milk proportion	Temperature	Time	Tryptose	CO_2	Skim milk (vol)
1	1	1	1	1	1	1	1	1
2	1	1	2	2	2	2	2	2
3	1	1	3	3	3	3	3	3
4	1	2	1	3	3	1	2	2
5	1	2	2	1	1	2	3	3
6	1	2	3	2	2	3	1	1
7	1	3	1	2	3	2	1	3
8	1	3	2	3	1	3	2	1
9	1	3	3	1	2	1	3	2
10	2	1	1	2	1	3	3	2
11	2	1	2	3	2	1	1	3
12	2	1	3	1	3	2	2	1
13	2	2	1	3	2	2	3	1
14	2	2	2	1	3	3	1	2
15	2	2	3	2	1	1	2	3
16	2	3	1	1	2	3	2	3
17	2	3	2	2	3	1	3	1
18	2	3	3	3	1	2	1	2

MATERIALS AND METHODS

Different media including skim milk [8], low fat milk, full fat milk and MRS (casein peptone, 10.0 g; meat extract, 10.0 g; yeast extract, 5.0 g; glucose, 20.0 g; K_2HPO_4 5.0 g; diammonium citrate, 2.0 g; Na acetate 5.0 g; $MgSO_4.7H_2O$, 0.5 g; $MnSO_4$. $4H_2O$, 0.2 g; Tween 80, 1 g; agar, 15.0 g; distilled in water, 1000 ml; pH= 6.2-6.4 sterilized at 121°C for 15 min) were used to produce kefir grains. The kefir was precipitated by centrifuge for 2 minutes.

The Taguchi method [9] was employed and the set of experiments were designed by Taguchi method. In each set, 8 variables were considered, one in two Level and others have 3 Levels. Levels introduced quantitative amount of a variable in experiments. Table 1 and table 2 show the variable factors and the Levels of Taguchi experimental design L-18, respectively.

Different Gram positive (*Bacillus, Corynebacterium, Listeria, Staphylococcus*) and Gram negative *(E.coli, Kelebsiela, Pseudomonas)* were grown on LB medium and inoculated on Nutrient Agar. Kefir grains were embedded over the culture. The Plates were incubated at 37°C for 24 hr. The zone of inhibition for growth was studied to make sure that produced kefir grains are active.

RESULTS AND DISCUSSION

Kefir grains are the natural starter used during kefir production. Factors influencing grain weight such as incubation temperature of 18, 22, 25 or 30°C, enrichment with combinations of tryptose (20 g/L) and yeast extract (20 g/L) and cultivation with or without agitation were studied by Schoevers to develop a method for mass grain production. The highest grain production was found by

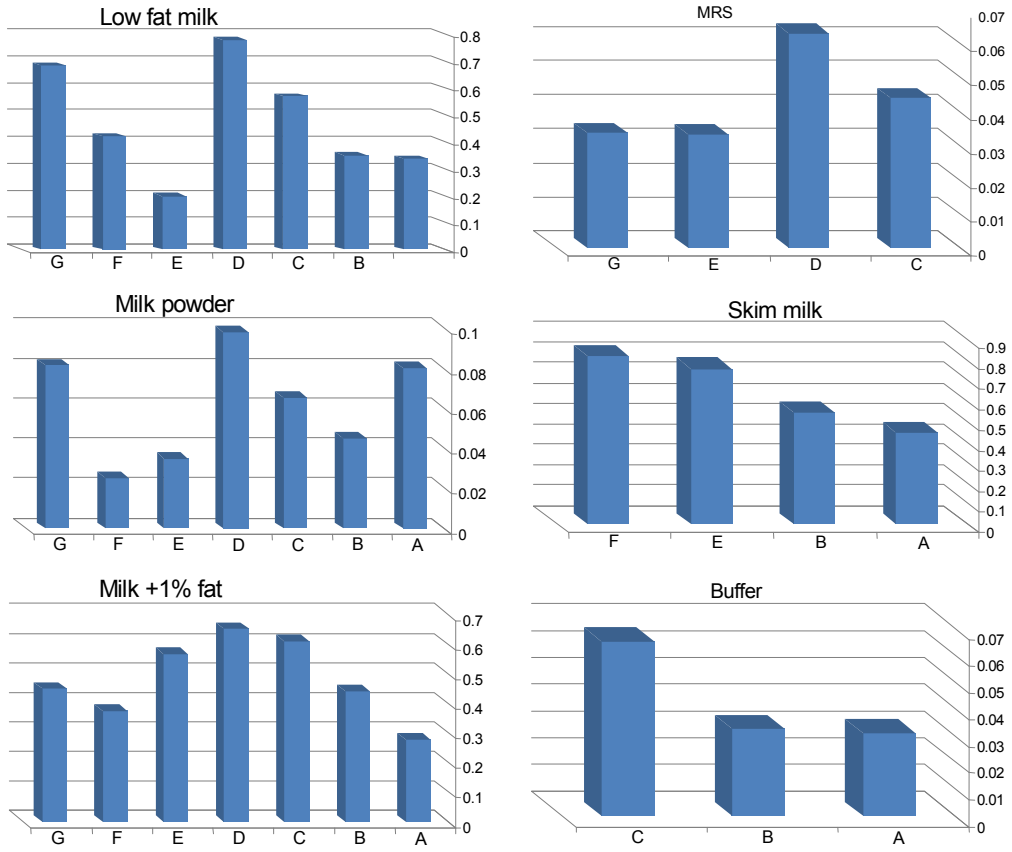

Fig. 1: Comparison of different milk containing media to produce kefir grains, as it is shown the grain is not produced in MRS and milk powder, however low fat milk and skim milk is the best media to produce kefir grain in minitube incubated for 24 hr. A= Aerobic pasteurized 37°C, B=Anaerobic pasteurized 37°C, C=Aerobic Sterilized 37°C, D= Anaerobic Sterilized 37°C, E = Aerobic Sterilized, 25°C, F= Aerobic Pasteurized 25°C and G= Pasteurized +Shaking 25°C.

them with activated grains cultivated with agitation at 25°C in low-fat milk containing tryptose and replacing the fermented milk daily. In our work, preliminary experiments have done to select the best media for kefir grain production by mini-fermentation tubes. Results are shown in figures 1 and 2. As it is shown among low fat milk (1%) full fat milk (3%) and skim milk, the latest was the best media to produce kefir grain in mini-tube. These results were obtained by others too [6]. However, here we used mini tube to produce kefir grain. The antibacterial effect of kefir obtained from mini-fermentation was studied to make sure that produced kefir grains are active. The zone of inhibition for growth was observed in *Corynebacterium* (Gram positive) and *listeria* (Gram positive). These results showed that the kefir produced in mini tube has also antimicrobial effect (Figure 3). This result is in accordance with that of Farnworth [10].

There are a number of environmental and biological parameters that effect on fermentation process. Design experiment helps us to study many variables simultaneously and most economically. By studying the effects of individual factors on the results, the best factor combination can be determined. In this work the Taquchi method has been used to optimize conditions to produce kefir grains in skim milk. The results are shown in tables (3-5). As it is shown in table 3, the maximum kefir grain production (0.85 mg) is produced in experiment 3 with 1.5 ml of 10% skim milk. Also minimum kefir grain production (0.110 mg) is obtained with 2% skim milk and 0.5ml skim milk volume. It can be clearly concluded from table 3 the kefir grain production is more in 10% skim milk and addition of fat (10 µl) significantly reduced the kefir grain from 0.25 to 0.428 g (Table 3).

Fig. 2: Production of kefir in minitube. A-Low fat milk, B-Skim milk, C-full fat milk,D-MRS media.All tubes incubated for 24 hr and washed two times. The kefir was precipitated by centrifuge for 2 minutes.

Fig. 3: Antimicrobial effect of Kefir grain. Zone of inhibition induced by kefir on *Corynebacterium*

The statistical analysis results of table 3 are given in table 4 and 5, by using the *Qualitek 4* software. The percent of contribution of each factor was determined by the analysis of variance (ANOVA). In addition to this analysis the optimum conditions could be estimated by the Taguchi method. In table 4 the p is the effect percent and shows the importance of each parameter in comparison of all parameters. As an important advantage

Table 3: Kefir grain production (g)

	Biomass of kefir grain	
Number of experiment	Repeat 1	Repeat 2
1	0.42	0.80
2	0.287	0.237
3	0.810	0,892
4	0.154	0.191
5	0.160	0.143
6	0.433	0.466
7	0.164	0.151
8	0.181	0.166
9	0.434	0.519
10	0.109	0.111
11	0.272	0.225
12	0.479	0.307
13	0.171	0.159
14	0.370	0.329
15	0.269	0.201
16	0.133	0.102
17	0.417	0.402

of the Taguchi method its ability to calculate the percent effect of missed variables or parameters in experiments. In table 5 the optimum condition of each parameter are shown. The multiple graphs of main effects are shown in figure 4. As it is shown in table 5 glucose and fat in level 3 had negative effect on kefir production, however time, temperature skim milk concentration, volume of skim milk and CO_2 in level 3 had positive effect on kefir grain production. The ANOVA analysis for kefir grain production in mini tube is shown in table 4. As it is shown the effect of skim milk is 46.17 and the volume of skim milk is 24.65 percent. However the effect of temperature from 25-37°C is only 5.9% and although fat inhibits kefir production but its effect is only 1.8%.

Taguchi L18 experiments showed that low concentration of the additive yeast and glucose did not have any significant effect on kefir grain production. Another Taguchi L9 experiment were designed with 3 factors: glucose (0.5, 1 and 1.5%),

Table 4: The percentage contribution of each factor determined by analysis of variant (ANOVA)

Factors	Level	Sum of squer	Varience	F-Ratio	Pure sum	Percent P(%)
Fat	1	0.26	0.26	8.295	0.23	1.898
Glucose	2	0.32	0.16	5.012	0.25	2.082
Skim milk	3	578.00	289.00	89.750	572.00	46.179
Temperature	3	0.80	0.40	13.472	0.73	5.969
Time	3	124.00	0.62	19.265	117.00	9.503
Tryptose	2	0.03	0.01	61.000	0.00	0.000
CO_2	2	0.16	0.8	2.546	0.09	8.400
Skim milk vol	3	311.00	155.00	48.380	305.00	24.653
Error						8.907

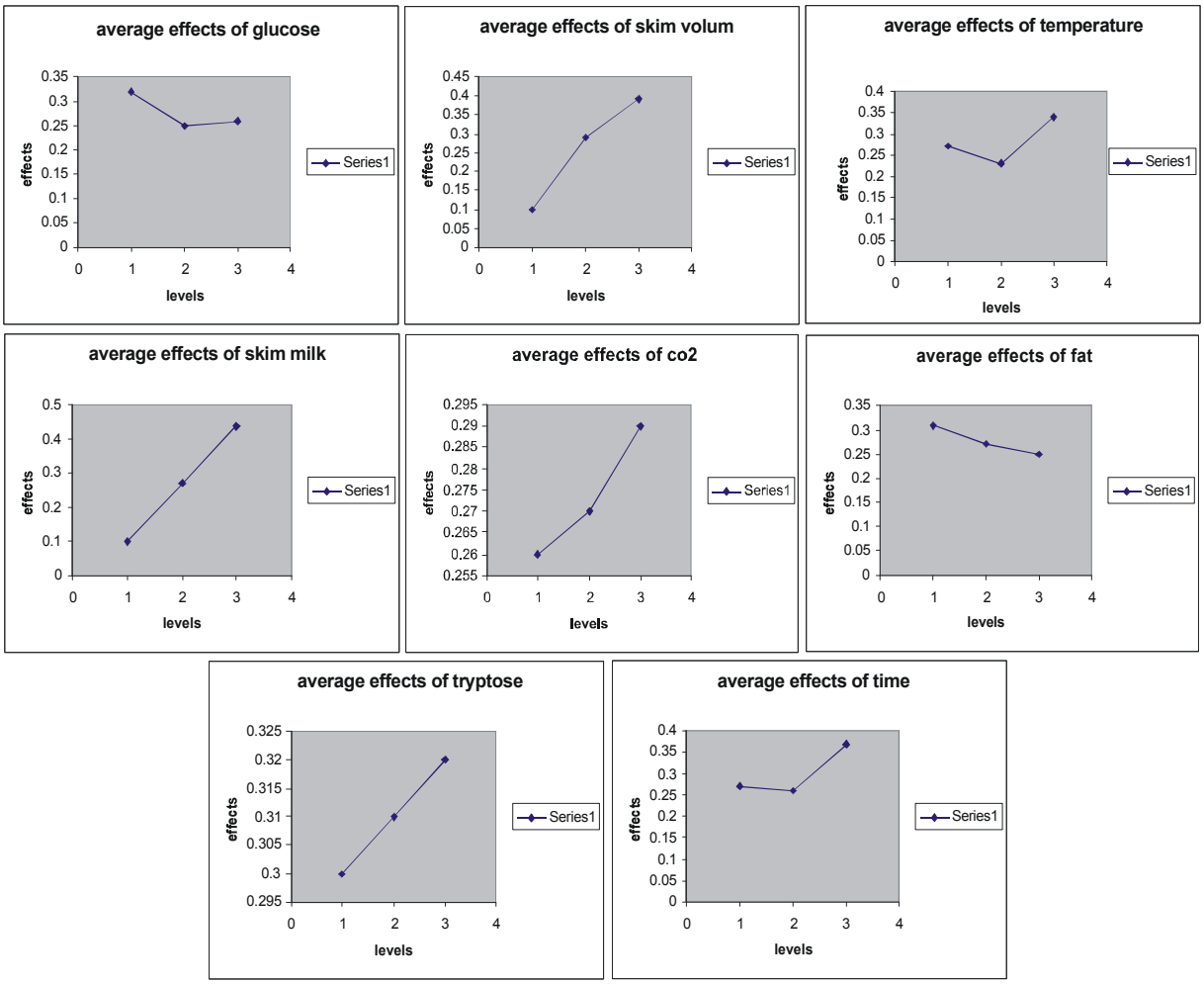

Fig. 4: Average effects of eight factors in three levels on production of kefir grain obtained by Taghuchi method. As it is shown volume and concentration of skim milk are the most effective parameters.

Table 5: Optimum condition for maximum kefir grain production

Factors	Optimum condition
Fat	0
Glucose	0.01%
Skim milk	10%
Temperature	37°C
Time	36 hr
Tryptose	0%
CO_2	10%
Skim milk	1.5ml

yeast extract (1,2 and3%) and different concentration of skim milk (9, 11 and 13%). In this experiment the optimum condition of kefir grain production were 13% skim milk 1.5% glucose and 1% yeast extract. ANOVA analysis for this experiment showed that higher concentration of skim milk significantly increased kefir grain production with 88% effect. With higher concentration of skim milk, the effect of yeast extract (1%) and glucose (1.5) are low but effective. However, by having 13% skim milk the effect of addition of glucose and yeast extract were 34 and 12%, respectively.

In conclusion optimum conditions were obtained for kefir grain production by a DOE using Taquchi method. The most important parameters are the relation between the inoculums volume and percentage of skim milk. Also it can be concluded that CO_2, incubation time and temperature and fat are effective parameters.

REFERENCES

1. La Rivière, J.W.M., P. Kooiman and K. Schmidt, 1967. Kefiran, a novel polysaccharide produced in the kefir grain by Lactobacillus brevis. Archive für Mikrobiologie, 59: 269-278.

2. Steinkraus, K H., 1996. Acid-fermented milk and milk/ cereal foods Handbook of Indigenous Fermented Foods 2nd edn. New York: Marcel Dekker, pp: 305-308.
3. Libudzisz, Z. and A. Piatkiewicz, 1990. Kefir production in Poland. Dairy Industries International, 55: 31-33.
4. Pidoux, M., 1988. Microflora of sugary Kefir. World J. Microbiol Biotechnol., 5: 223-238.
5. Mainville, I., R. Normand, L. Byong and RE. Farnworth, 2006. Polyphasic characterization of the lactic acid bacteria in kefir. *Systematic and Applied Microbiology,* 26: 59-68.
6. Schoevers, A. and T.J. Britz, 2003. Influence of different culturing condition on Kefir grain increase. International J. Dairy Technol., 56: 183-187.
7. Gracielal, G., A. Abraham and G.D. Antoni, 1998. Characteristics of Kefir prepared with different grain: milk ratio. J. Dairy Res., 65: 149-154.
8. Beshkova, D.M., E.D. Simova, Z.I. Simov, G.I. Frengova and Z.N. Spasov, 2002. Pure cultures for making kefir. Food Microbiol., 19: 537-544.
9. Ranjit, K.R., 2001. Design of experiments using the Taguchi approach: 16 steps to product and process improvement. *Wiley* New York.
10. Farnworth, R.E., 2005. Kefir- a complex probiotic. Food Science and Technol., 2: 1-17.