

Optimization of non-nutritional factors for enhancement of nisin production

Nisin, has been used as a food biopreservative for over forty years in more than 80 countries including the United States and Europe and is produced by *Lactococcus lactis*, a bacterium used widely for industrial production of fermented dairy products such as milk, cheese, and yogurt. In order to increase nisin production by the bacterium, non-nutritional factors as well as nutritional parameters must be optimized. Optimization is defined as: an act, process, or methodology of making something (as a design, system, or decision) as fully perfect, functional, or effective as possible; specifically: the mathematical procedures for finding the maximum of a function. In other words, optimization is the selection of a best element (with regard to some criteria) from some set of available alternatives.

Trial conditions number	Temperature (°C)	Agitation (rpm)	Inoculum age (h)	Inoculum size (%)	pH	Flask volume/medium volume ratio	Maximum nisin production (IU/ml)	Maximum nisin productivity (IU/ml/h)
1	27	0	24	1.0	6.00	10.00	291.29	15.88
2	27	0	32	5.0	7.00	5.00	440.94	22.00
3	27	0	40	10.0	8.00	3.33	410.22	20.13
4	27	100	24	1.0	7.00	5.0	534.72	26.15
5	27	100	32	5.0	8.00	3.33	453.62	19.10
6	27	100	40	10.0	6.00	10.00	236.83	9.30
7	27	200	24	5.0	6.00	3.33	179.95	6.66
8	27	200	32	10.0	7.00	10.00	199.93	7.17
9	27	200	40	1.0	8.00	5.00	260.01	13.11
10	30	0	24	10.0	8.00	5.00	169.96	7.09
11	30	0	32	1.0	6.00	3.33	159.92	4.89
12	30	0	40	5.0	7.00	10.00	157.96	8.12
13	30	100	24	5.0	8.00	10.00	518.53	24.43
14	30	100	32	10.0	6.00	5.00	294.10	11.94
15	30	100	40	1.0	7.00	3.33	449.28	18.74
16	30	200	24	10.0	7.00	3.33	501.28	22.92
17	30	200	32	1.0	8.00	10.00	564.96	23.54
18	30	200	40	5.0	6.00	5.00	361.64	11.24

Fig. 1. The factors in orthogonal array design with maximum nisin production and productivity as results of the trial conditions.

In the present study, optimization of temperature, agitation, age and size of inoculum, medium initial pH value and flask volume/medium volume ratio (F/M ratio) in de Man, Rogosa and Sharpe (MRS) culture medium using orthogonal array method (Qualitek-4 software, Nutek, Inc., Version 6.2.0, USA) in order to enhancement of nisin production were accomplished. Orthogonal array method is a kind of statistical design of experiment which is often employed in industrial experiments to study the effect of several control factors. Nisin was produced by *Lactococcus lactis* subsp. *lactis* PTCC 1336 and measured by bioassay method using *Micrococcus luteus* PTCC 1169 as the nisin-sensitive strain.

Fig. 1. shows the values of the factors in orthogonal array design with nisin maximum production and productivity as the results of the trial conditions. In order to obtain optimum non-nutritional factors for nisin production, the results of maximum nisin production and maximum nisin productivity of each trial condition were subjected to analysis by the software. For verification tests, in addition to standard analysis (using average of the results), a signal/noise (S/N) ratio analysis (using mean-squared deviation or MSD), was performed. The optimum non-nutritional factors level of verification tests, the results at the optimum levels estimated by the software, and the experimental results are shown in Fig. 2.

Verification tests fermentation conditions	Temperature (°C)	Agitation (rpm)	Inoculum age (h)	Inoculum size (%)	pH	Flask volume/m edium volume ratio	Maximum nisin production (IU/ml)		Maximum nisin productivity (IU/ml/h)	
							estimated by the software	obtained by experiment	estimated by the software	obtained by experiment
Maximum production (Standard analysis and S/N Ratio analysis)	30	100	24	1.0	8.00	3.33	554.26	606.72	-	-
Maximum productivity (Standard analysis)	27	100	24	1.0	8.00	3.33	-	-	25.65	28.76
Maximum productivity (S/N Ratio)	27	100	24	1.0	8.00	5.00	-	-	29.16	37.48

Fig. 2. The non-nutritional factors levels of verification tests, estimated and experimental results.

According to the experimental results, the optimum levels of non-nutritional factors for maximum nisin production and productivity were obtained as: flask volume/medium volume ratio: 5.00, medium initial pH value 8.00, inoculum size: 1 %, inoculum age: 24-hour-old (A=1.7), agitation: 100 rpm and temperature: 27 °C. Under the optimized conditions, maximum nisin production and maximum nisin productivity were 599.70 IU/ml and 37.48 IU/ml/h respectively. Whereas all researchers have used 30 °C for nisin production studies, interestingly after optimization, maximum nisin production and production productivity were observed at 27 °C.

The study showed that nisin production was strongly influenced by factors such as agitation, initial pH value of the medium and inoculum size. Neutral to slightly alkaline initial pH value of the medium, lower inoculum size and moderate agitation were the best conditions for higher nisin production and productivity. It was concluded from the results of verification tests that decrease in temperature and increase in flask volume/medium volume ratio play an important role in maximum nisin production and productivity.

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Publication

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