Advance Journal of Food Science and Technology 10(10): 750-758, 2016
DOI: 10.19026/ajfst.10.2256
ISSN: 2042-4868; e-ISSN: 2042-4876

Submitted: June 8, 2015 Accepted: July 14, 2015 Published: April 05, 2016

Research Article

Optimization of Different Medical and Edible Plant Formulation in Growth-Promoting Effects for *L. Bulgaricus* using Mixture Design

Yamei Yan, Jiali Lv, Xiaopeng Wang, Bing Du and Wenjuan Li
School of Food and Biological Engineering, Shaanxi University of Science and Technology, Xi'an, China

Abstract: To improve the activity of probiotics and take advantage of abudant medical and edible plants in China, 18 kinds of medical and edible plants which were chosen to study growth-promoting effects for *L. Bulgaricus* by single factor, 8 kinds of plants that have significant growth-promoting effect through preliminary screening have obtained. On the basis of this, the different combinations which study growth-promoting effects to *L. Bulgaricus* using simplex-centroid experimental design and the viable number was chosen as detection index. The result shows that the medical and edible plants of obvious growth-promoting effects are Coix seed, Lotus seeds, Wheat germ, Chinese yam, Pueraria, Lilium, Mulberry, Jujube. The optimal combination according to simplex-centroid experimental design is Coix seed 5.507%, Lotus seeds 30.834%, Wheat germ 20.545%, Chinese yam 20.545%, Pueraria 4.739%, Lilium 15.259%, Mulberry 6.321%, Jujube 6.321%, the viable number is up to 3.89×10^8 cfu/mL.

Keywords: Formulation, growth, *L. Bulgaricus*, medical and edible plant, mixture design

INTRODUCTION

The medical and edible plants are kinds of herds containing complex ingredients (Chui, 1997; Zhang *et al*., 2006) (sugars, tannins, resins, pigments, amino acids, volatile oils, glycosides, alkaloids and inorganic compositions) which can both be used as food and medicine (Wang *et al*., 2004; Jiang *et al*., 2015). The Chinese scientists have studied and used the medical and edible plant for thousands of years, but there are still some scientific questions to be solved. Probiotics are kinds of microorganisms that play important role in human health with adequate amounts (FAO/WHO, 2002; Guarner and Schaafsma, 1998). Researchers have shown that the medical and edible plants could promote the growth of probiotics. A review concerning the growth-promoting factors (traditional Chinese drug and other substances) of probiotics is presented. The mechanism of growth promoting was primarily analyzed and the direction of research has been brought forward. The review is helpful to improve the number of probiotics in products, provide thought for modernization of Chinese herbal drug and develop functional food (Shu *et al*., 2009). Effects of adding different nutrients in jerusalem artichoke medium on the growth of *Lactobacillus bulgaricus* (L.b-S1) were examined. Furthermore, the optimum enrichment medium of L.b-S1 were screened by orthogonality test. (Li *et al*., 2012).

In present work, the plants used in this experiment to culture *L. Bulgaricus* are costed in low and easy to find. Furthermore, after the preliminary screening, 8 kinds of medical and edible plant among the 18 kinds were chosen for their significant growth-promoting effect. The Chinese herbal drug which were used in this experiment to culture *L. Bulgaricus* is cheap and easy to find. The one which had the best growth promoting potential for *L. Bulgaricus* was chosen for further study. 18 kinds of medical and edible plant which was chosen study growth-promoting effects for *L. Bulgaricus* by single factor, 8 kind of medical and edible plant medium that have significant growth-promoting effect through preliminary screening have obtained. Base on previous study, the experiment used to study the growth-promoting effects to *L. Bulgaricus* using simplex-centroid experimental design, the viable number was chosen as detection index. In order to further improve the viablity of the probiotics, the modernization of Chinese medicine and the development of probiotics related products.

MATERIALS AND METHODS

Bacterials and culture conditions: *L. Bulgaricus* (LB) were kindly provided by School of Food and Biological Engineering, Shaanxi University of Science and Technology.

(LB) inoculated was cultured in MRS broth medium as activation culture medium by 1%(v/v) ratio at 37°C for 48 h under an anaerobic atmosphere.

Corresponding Author: Jiali Lv, School of Food and Biological Engineering, Shaanxi University of Science and Technology, Xi'an, China

Plant materials: The 18 kinds of medical and edible plant used in this experiment were Plum, Hawthorn, Coix seed, Lotus seeds, Wheat germ, Chinese yam, Papaya, Arrowroot, Lilium, Light bamboo, Basil, Poria, Rhizoma Imperatae, Mulberry, Bergamot, Polygonatum, Jujube, Longan.

Preliminary screening of the medical and edible plant medium: In the Pre-Process, appropriate amount of plant medical and edible was pulverized in high-speed grinder with 40 mesh sieve. Mixture of water and the powder of medical and edible plant in a ratio of 1/10, (Guarner and Schaafsma, 1998) sterilization 120°C, 20 min. *L. Bulgaricus* (LB) was respectively inoculated in medical and edible plant medium by 2% were used as control mediums. Cultured in 37°C, 48 h. The viable number was measured after 48 h.

Optimization of different medical and edible plant formulation using mixture design: Based on the preliminary screening of the single factor, 8 kind of plant medical and edible (A Coix seed, B lotus seeds, C Wheat germ, D Chinese yam, E Pueraria, F Lilium, G Mulberry, H jujube) was complexed in the method of simplex-centroid experimental design in mixture design (Ying *et al*., 2014) and 280 groups of experimental groups were obtained, according to the above methods, The optimum composition was selected.

Statistical analysis: The simplex-centroid experimental design, statistical analysis and model performed by the Design Expert (Version7.0, State-Ease InC, Minneapolis, MNUSA) to study the best ratio of ingredients which evaluate each component of the formulation and their interactions and also can optimize composition in accordance with the target setting by establishing a continuous variable surface model. Then the experiment results were obtained.

RESULTS AND DISCUSSION

Preliminary screening of the medical and edible plant medium: The initial pH value of Plum, Hawthorn and Quince was less than 3.20, the pH value is also less than 3.50, however, the pH value of Light bamboo, Basil after 48 h was higher 5.5, it is not suitable for the growth of LB (Fig. 1).

Compared with the reference when fermented for 0 h, the viable number was more than 10^6 cfu/mL, after fermenting for 48 h, the viable number of Plum, Hawthorn, Papaya, light bamboo, Bergamot was less than 10^6 cfu/mL, the viable number of Lotus seeds, wheat germ, Chinese yam, Lilium, jujube viable cell count reached 10^7 cfu/mL. Base on the study, Coix seed, lotus seeds, Wheat germ, Chinese yam, Pueraria, Lilium, Mulberry and Jujube were chosen for further study (Fig. 2).

Optimization of different medical and edible plant formulation using mixture design:

The change of pH value: According to the above graphs, the initial pH value was in range of 5~7, the range of change was 3~4 when *L. Bulgaricus* (LB) was respectively inoculated in medical and edible plant medium by 2% after fermentation 48 h, it is closely related to the change of the pH value and viable number (Fig. 3 to 10).

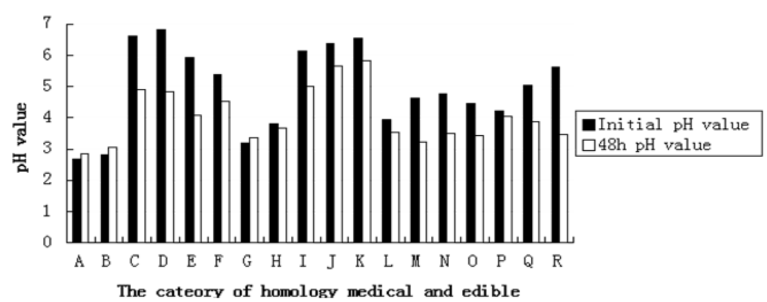


Fig. 1: The pH value of LB when grow in different plant medical and edible

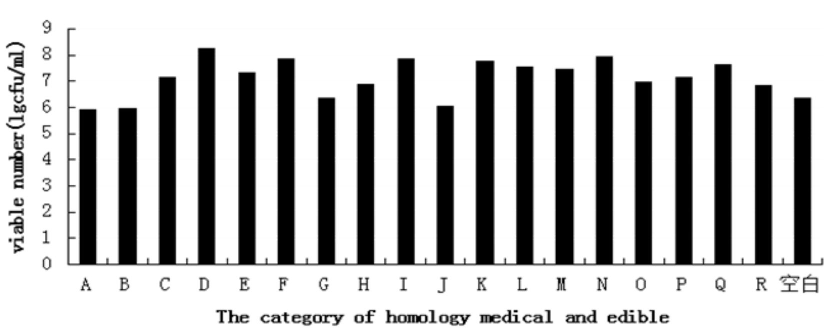


Fig. 2: The viable number of LB when grow in different medical and edible plant

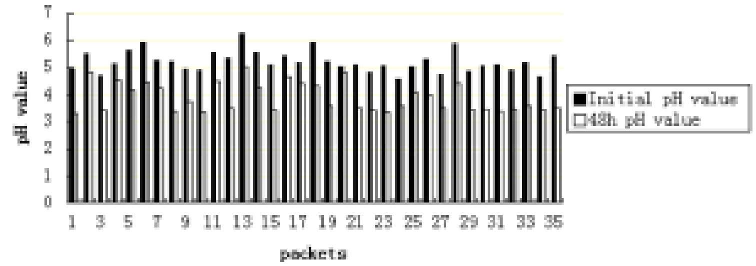


Fig. 3: The pH value of LB when grow 1-35 groups of different medical and edible plant formulation

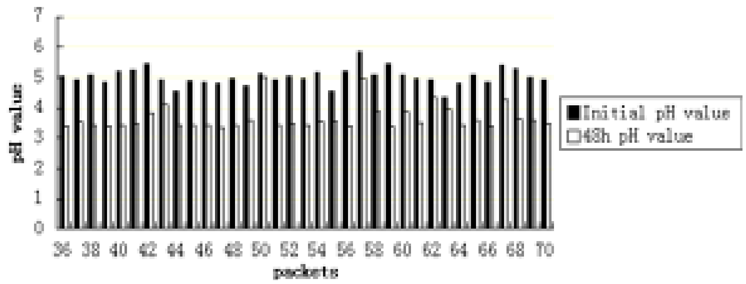


Fig. 4: The pH value of LB when grow 36-70 groups of different medical and edible plant formulation

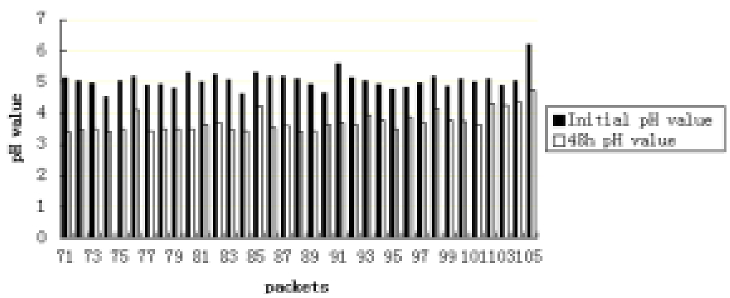


Fig. 5: The pH value of LB when grow 71-105 groups of different medical and edible plant formulation

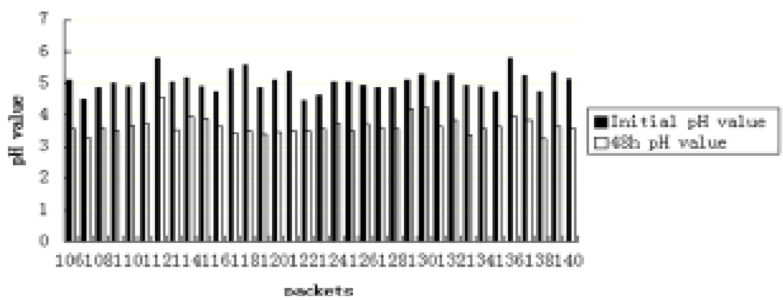


Fig. 6: The pH value of LB when grow 106-140 groups of different medical and edible plant formulation

Fig. 7: The pH value of LB when grow 141-175 groups of different medical and edible plant formulation

Fig. 8: The pH value of LB when grow 176-210 groups of different medical and edible plant formulation

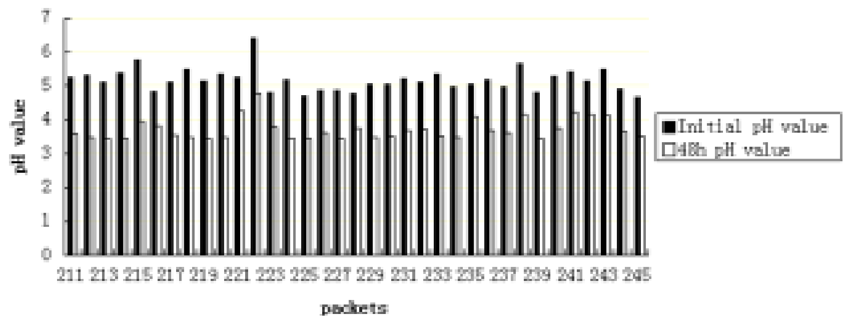


Fig. 9: The pH value of LB when grow 241-245 groups of different medical and edible plant formulation

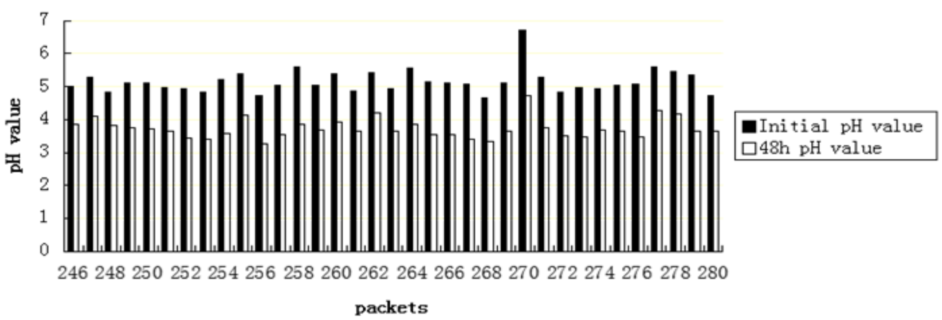


Fig. 10: The pH value of LB when grow 246-280 groups of different medical and edible plant formulation

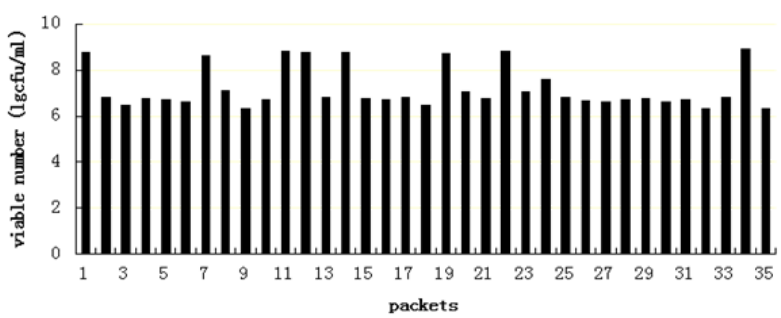


Fig. 11: The viable number of LB when grow 1-35 groups of different medical and edible plant formulation

The change of viable number: The viable number was more than 10^6 cfu/mL when fermented for 0 h, however, the viable number of *L. Bulgaricus* was range from 10^6 cfu/mL to 10^8 cfu/mL in different plant formulation after fermenting for 48 h, (Fig. 11 to 18). Therefore, the viable number of 280 plant formulation as an important index was chosen which analyzed using the software according to the below methods.

Regression equation: 8 plants were complexed according to the simplex-centroid experimental design in mixture design, the range of different components is

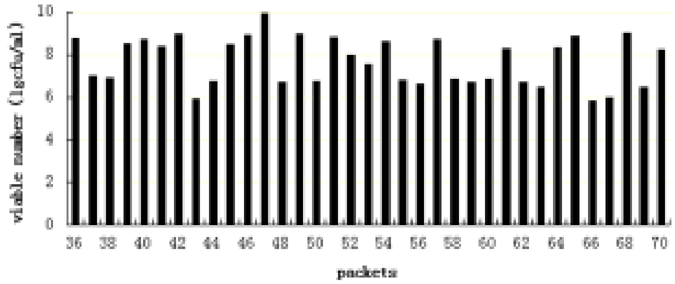


Fig. 12: The viable number of LB when grow 36-70 groups of different medical and edible plant formulation

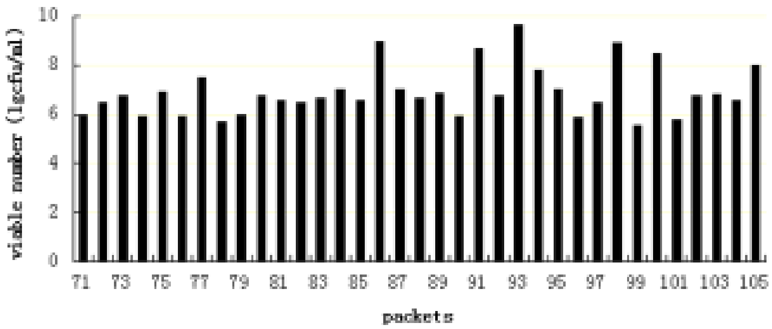


Fig. 13: The viable number of LB when grow 71-105 groups of different medical and edible plant formulation

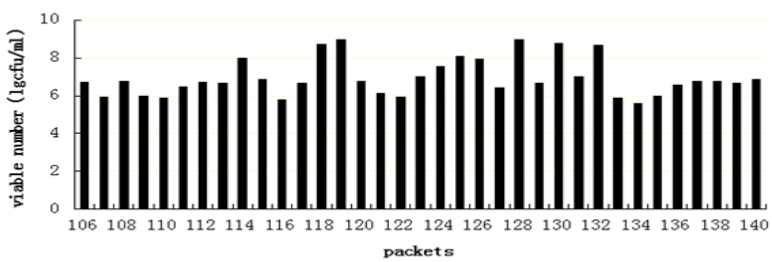


Fig. 14: The viable number of LB when grow 106-140 groups of different medical and edible plant formulation

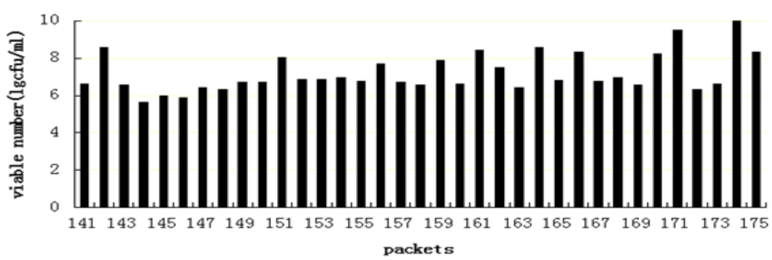


Fig. 15: The viable number of LB when grow 141-175 groups of different medical and edible plant formulation

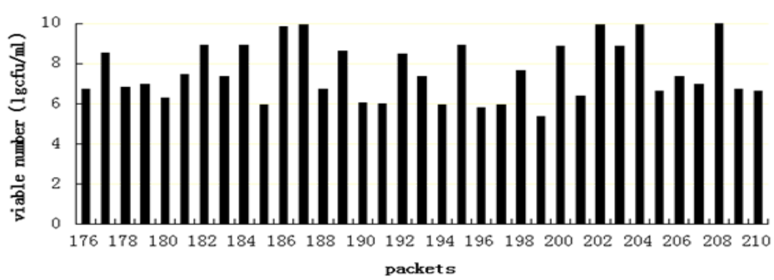


Fig. 16: The viable number of LB when grow 176-210 groups of different medical and edible plant formulation

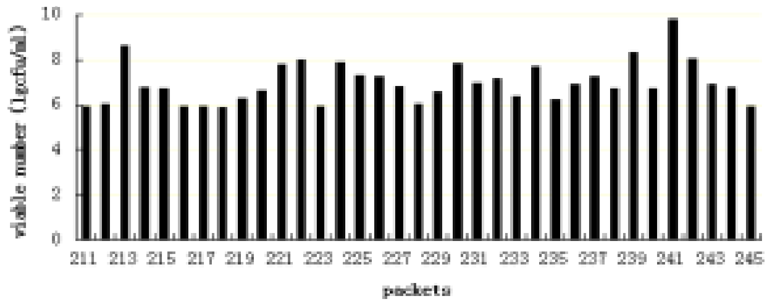


Fig. 17: The viable number of LB when grow 211-245 groups of different medical and edible plant formulation

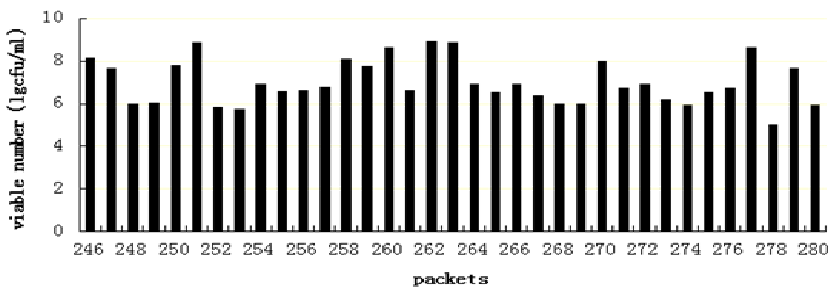


Fig. 18: The viable number of LB when grow 246-280 groups of different medical and edible plant formulation

Table 1: The sensory scores of quadratic regression model on variance analysis

Source	Sum of squares	df	Mean Square	F-value	p-value
Block	28.67	12	2.39		
Model	26.03	7	3.72	3.57	0.0011
Linear	26.03	7	3.72	3.57	0.0011
Residual	270.59	260	1.04		
Lack of fit	270.59	255	1.06		
Pure error	0	5	0		
Cor Total	325.3	279			
R^2 = 0.8786					

Table 2: The target range of experimental variable an response value

Name	Component	Goal	Low level	High level
Coix seed	A	In range	0	100
Lotus seeds	B	In range	0	100
Wheat germ	C	In range	0	100
Chinese yam	D	In range	0	100
Pueraria	E	In range	0	100
Lilium	F	In range	0	100
Mulberry	G	In range	0	100
Jujube	H	In range	0	100
Viable number	R	Maximize	5.5×10^8	8.51×10^9

$0 \le xi \le 100$. With the software, the final regression equation for the experimental value of the viable number which performed quadratic regression fit is:

$$R = +0.073230 \times \text{Coix seed} + 0.085425 \times \text{Lotus seeds} + 0.077313 \times \text{Wheat germ} + 0.065111 \times \text{Chinese yam} + 0.064582 \times \text{Pueraria} + 0.075117 \times \text{Lilium} + 0.075998 \times \text{Mulberry} + 0.071611 \times \text{Jujube} + 6.32442\text{E-}004 \times \text{Lotus seeds} \times \text{Lilium}$$

Through the variance analysis the of the model equation showed that the quadratic model of the sensory scores were significant ($p<0.001$), the coefficient of determination R^2 is 0.8786, therefore, variation Y (A, B, C, D, E, F, G, H) is caused by 88% of variables, the result shows that Model equations can fit well with the ratio relationship index and formulation and can be used to predict the viable number (Table 1).

The change of each component for the effects of viable number: In accordance with the response surface graph and the three contour map, how the viable number be effected by the change of each component of medical and edible plant is visible. The formula in this research totally contains 8 components. The effects of the interaction of Coix seed, Lotus seeds and Wheat germ on viable number. On the center of gravity location, the content of Coix seed, Lotus seeds and Wheat germ is 12.5%, viable number is 2.1×10^7 cfu/mL. On the point where the content of Lotus seeds is 37.5% and Coix seed and Wheat germ are at low level, the viable number rise to 3.5×10^7 cfu/mL (Fig. 19); the effects of the interaction of Wheat germ, Chinese yam and Pueraria on viable number. When Wheat germ is at a high level of content, the viable number approaches to 1.0×10^8 cfu/mL. On the center of gravity location, the content of Wheat germ, Chinese yam and Pueraria is 12.5%, the viable number is 2.1×10^7 cfu/mL (Fig. 20). The effects of the interaction of Lilium, Mulberry and Jujube on viable number. The higher the percentage of Lilium, the faster viable number increases. And when it reaches 27.6%, the viable number can be 2.72×10^7 cfu/mL. However, the viable number reaches its minimum at the point where content of Jujube is 37.5% (Fig. 21).

The optimization experiments of the formulation: The range and desired response of various components were setted up by optimization and analysis function of the software (Table 2), then running the software. 6 kinds of combinations were obtained which were up to the target response value and provided a prediction (Table 3). The optimal combination is Coix seed 5.507%, Lotus seeds 30.834%, Wheat germ 20.545%, Chinese yam 20.545%, Pueraria 4.739%, Lilium 15.259%, Mulberry 6.321%, jujube 6.321%, the maximum viable number is 3.89×10^8 cfu/mL.

Table 3: The optimal combination and prediction result of different components

Number	Coix seed	Lotus seeds	Wheat germ	Chinese yam	Pueraria	Lilium	Mulberry	Jujube	Viable Number (cfu/mL)
1	5.507	30.834	20.545	15.129	4.739	15.259	6.321	1.666	3.89×10^8
2	3.281	42.269	6.322	10.232	0	22.451	12.199	3.246	2.44×10^8
3	1.28	38.956	10.125	7.641	0	38.716	1.048	2.234	2.12×10^8
4	0	49.737	17.853	0	4.768	18.464	3.947	5.231	1.91×10^8
5	0	51.781	8.392	7.252	0	16.98	11.239	4.356	1.37×10^8
6	0	37.447	18.611	12.296	2.107	3.036	21.646	4.857	1.05×10^8

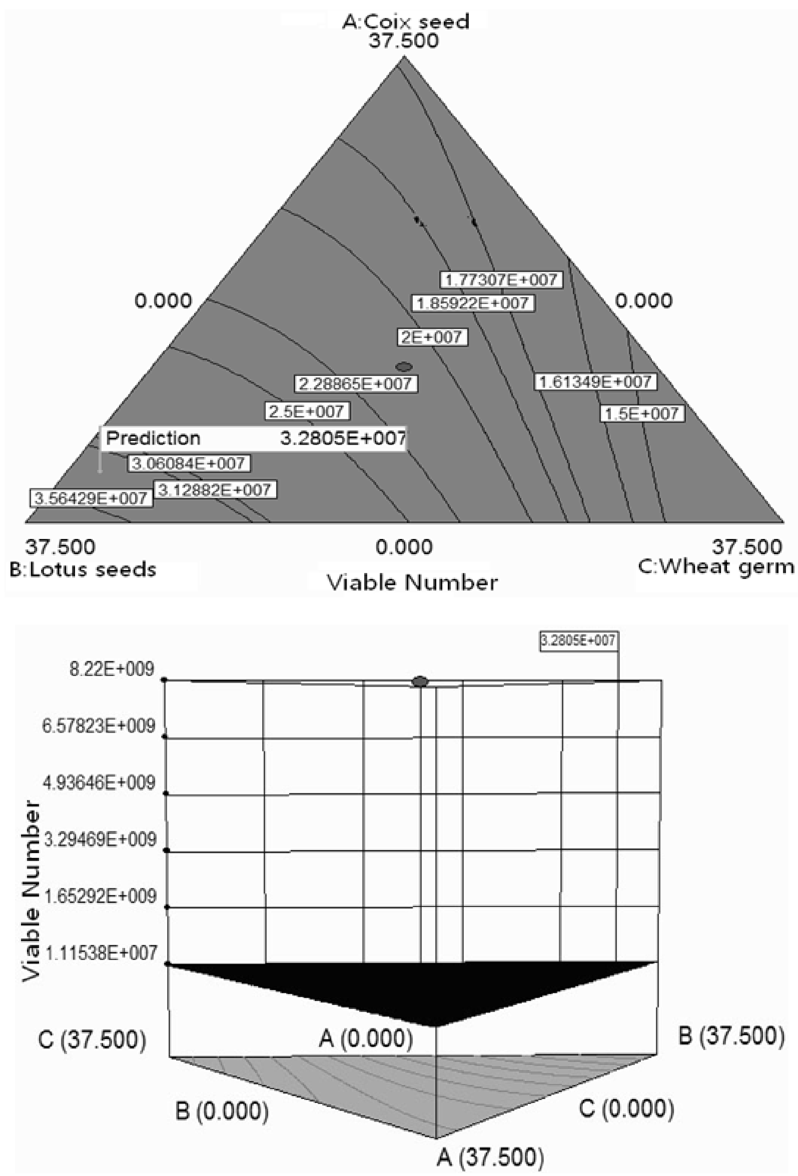


Fig. 19: The effects of viable number for the interaction of Coix seed/Lotus seeds and Wheat germ

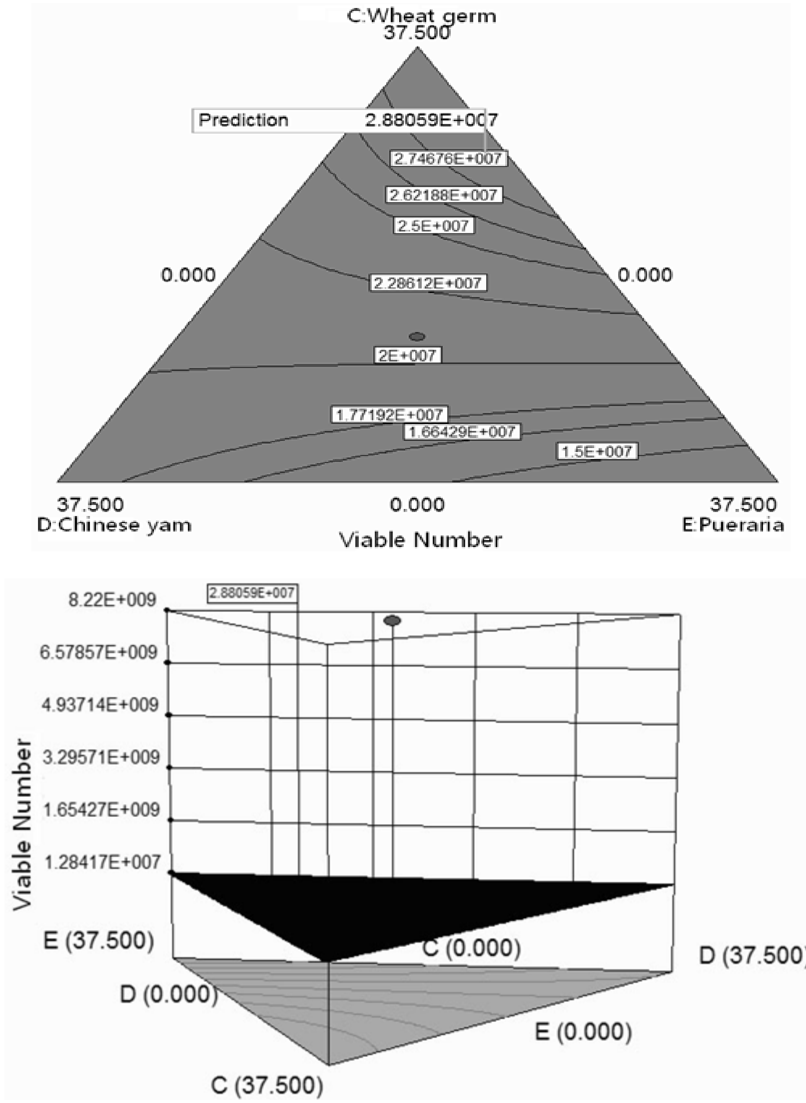
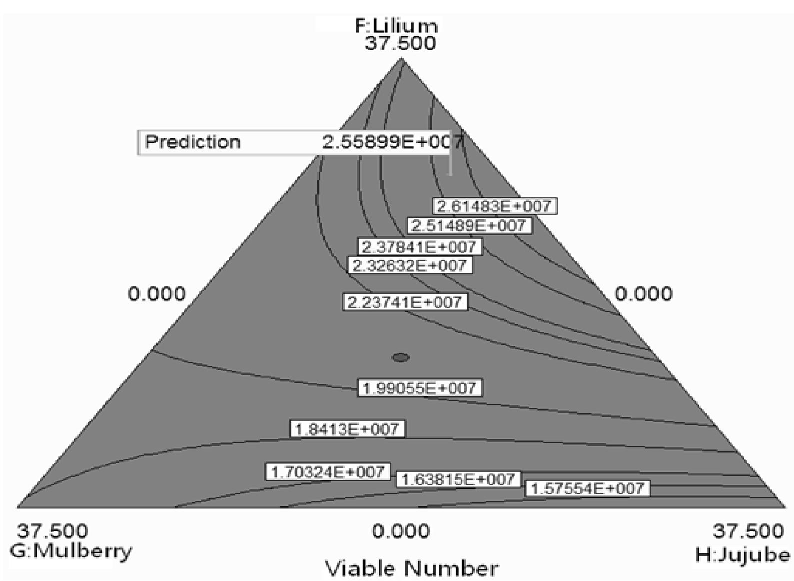


Fig. 20: The effects of viable number for the interaction of Wheat germ/ Chinese yam and Pueraria



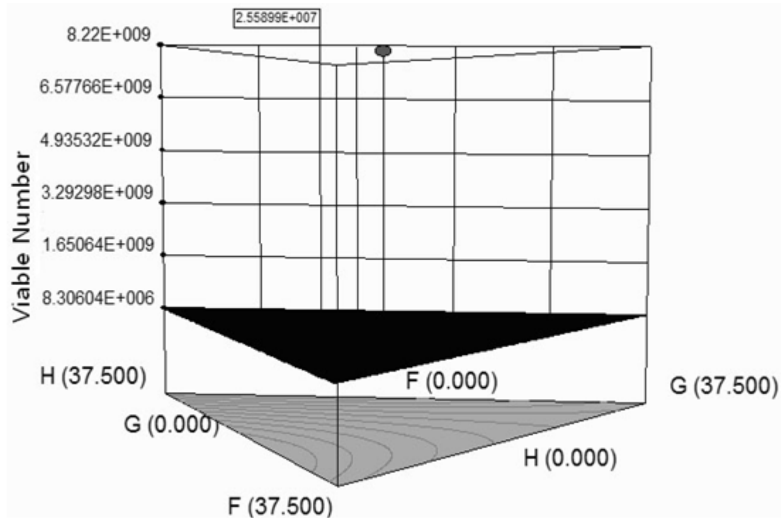


Fig. 21: The effects of viable number for the interaction of Lilium/Mulberry and Jujube

CONCLUSION

Eighteen (18) kinds of medical and edible plants which were chosen to study growth-promoting effects for *L. Bulgaricus* by single factor, the combination of 8 plants used in the study of growth-promoting effects for *L. Bulgaricus* adopted simplex-centroid experimental design, the viable number was chosen as a detection index. Simplex-centroid experimental design which evaluates each of the components in the formulation and their interactions and can optimize composition in accordance with the target setting by establishing a continuous variable surface model. The result shows that the 8 plants of obvious growth-promoting effects were chosen which were Coix seed, Lotus seeds, Wheat germ, Chinese yam, Pueraria, Lilium, Jujube, Mulberry by the change of pH value and the viable number. The initial pH value was in range of 5~7 and the range of change was 3~4 after fermentation for 48 h in 280 groups experimental groups, then the viable number of 280 plant formulation was chosen which analyzed using the software, finally, the growth of *L. Bulgaricus* was most evident when cultured in combination of Coix seed is 5.507%, lotus seeds 30.834%, Wheat germ 20.545%, Chinese yam 20.545%, Pueraria 4.739%, Lilium 15.259%, Mulberry 6.321%, jujube 6.321%, the highest number of viable cells of *L. Bulgaricus* was up to 3.89×10^{8} cfu/mL, it is of great significance in developing abundant Chinese medicine resources and unique health care products in China. However, improving the viability of *L. Bulgaricus* is still a subject for research. Further study is needed in the future.

ACKNOWLEDGMENT

This study has been financially supported by the "13115" scientific and technological innovation projects of major science and technology of Shaanxi Province, China (No. 2009ZDKG-20). The science and technology co-ordinator innovation projects plan project of Shaanxi Province, China (No.2011KTCQ03-08).

REFERENCES

Chui, S.D., 1997. Big Encyclopedia of Chinese Traditional Medicine. Heilongjiang Science and Technology Press, Heilongjiang.

FAO/WHO, 2002. Guidelines for the evaluation of probiotics in food. Food and Agriculture Organization of the United Nations and World Health Organization. Working Group Report.

Guarner, F. and G.J. Schaafsma, 1998. Probiotics. Int. J. Food Microbiol., 39: 237-238.

Jiang, C.X., J.G. Cheng and T. Luo, 2015. Nutritional value analysis of medicinal and edible plant. J. Anhui Agri. Sci., 43(11): 282-284.

Li, C., X.X. Gu, J.J. Tian, H.T. Tian, W.T. Xu and Y.B. Luo, 2012. Screening and optimizing of *Lactobacillus bulgaricus* jerusalem artichoke compound enrichment medium. J. Chinese Inst. Food Sci. Technol., 12(5): 82-87.

Shu, G.W., J.L. Lv and H. Chen, 2009. Research development of extract of traditional Chinese medicine as growth-promoting factor of probiotics. Food Sci. Technol., 34(10): 162-165.

Wang, C.T., B.P. Ji and G.H. Zhu, 2004. Effects of growth and conservation of Lactic acid bacterium on five kinds of chinese herbal medicines. Chinese J. Microecol., 16: 75-76.

Ying, Y.L., J.Z. Zhou and W.Q. Wang, 2014. Application of mixture design in the recipe of instant coarse cereal breakfast powder. Food Sci. Technol., 39(05): 172-175.

Zhang, B.W., Z.H. Hao, J.J. Wang and Y.S. Song, 2006. Study on creative way of research and exploitation in edible biologic medicines. China Condim., 34(10): 162-165.