Food Sci. Technol. Res., **7** (1), 84–87, 2001

Optimum Condition of High Pressure Treatment for the Preparation of Lysozyme-Dextran Complex Found by Random-Centroid Optimization

Kenjin NAKAMURA, Satoshi HATANO, Akiyoshi MATSUOKA, Noboru FURUKAWA, Tsuyoshi TAKAHASHI and Yoshitada YAMANAKA

Department of Zootechnical Science, Tokyo University of Agriculture, 1737 Funako, Atsugi city, Kanagawa, 243-0034, Japan

Received August 17, 2000; Accepted November 25, 2000

The lysozyme-dextran complex (LDC) was prepared in the liquid state using high pressure treatment. This method was able to shorten the reaction period for the preparation of LDC and to suppress the formation of melanoidin, although the production rate of LDC was decreased. The maximum production rate of LDC was attained by 20 experiments using the random-centroid optimization (RCO) method. The optimum condition for the preparation of LDC was pH 4.5, 0.1 M NaCl, 192 MPa, 19.3°C and the treatment time was 88 min, under which one-tenth of the lysozyme formed the complex. Optimization by the RCO method was successful in this study, and is reasonably expected to be a practical and powerful tool in various research fields.

Keywords: lysozyme-dextran complex, high pressure, random-centroid optimization

The lysozyme-dextran complex (LDC), which was prepared from powdered hen egg-white lysozyme and dextran by the naturally occurring Maillard reaction in a controlled dry-heating state, has excellent antimicrobial activities against Gram-negative bacteria (Nakamura *et al*., 1990; Nakamura *et al*., 1991). Similar results were also confirmed in our laboratory (Nakamura., 1996). However, this procedure might be troublesome for the preparation of LDC. First, more than two weeks were required for the preparation. Second, the separation of LDC from the final reactant was laborious due to the formation of melanoidin which insolubilizes the browning reactants.

The glycosylation using a high pressure treatment was observed in the liquid state under the thermally controlled conditions in our previous study (Nakamura *et al*., 1997). This method was able to shorten the reaction period for the preparation of LDC and to suppress the formation of melanoidin under the thermal controlled liquid condition (Tamaoka *et al*., 1991), although the production rate of LDC was decreased. In this study, the random-centroid optimization (RCO) (Nakai, 1990) was introduced to search for the optimum condition for preparation of LDC. RCO is a computational global optimization method. The huge numbers of experiments, advanced mathematical knowledge, and skillful techniques for program operation are usually required to apply most of the global optimization methods such as genetic algorithm optimization (Visweswaran & Floudas, 1990) and level set optimization (Yassen, 1993). Global optimization by RCO has been advocated by Nakai *et al*. (Nakai, 1990; Nakai *et al*., 1998) to solve these difficulties. The RCO program can optimize 3 to 30 factors at one time without a large number of experiments. This optimization program has been applied to find the optimum composition of ingredients for high-fiber bread containing wheat bran (Kobayashi *et al*., 1997), the cooking conditions for Indica type rice (Nishimura *et al*., 1997), the preparative method for cream puff paste (Nishimura *et al*., 1998) and maximizing the gel strength of carp (*Cyprinous carpio*) actomyosin (Nakai *et al*., 1999). The RCO program has been opened on a website (Nakai *et al*., 1998).

In this study, RCO was attempted to maximize the production rate of LDC in the liquid state using high pressure treatment.

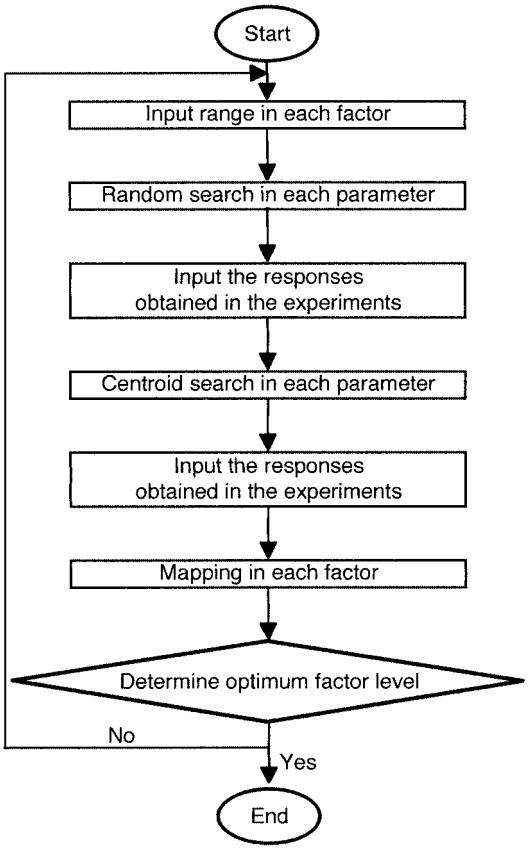

Fig. 1. Flowchart of random-centroid optimization.

E-mail: kenjin@nodai.ac.jp

Table 1. The range for each factor to optimize by random-centroid optimization to maximize the production rate of lysozyme-dextran complex in the liquid state using high pressure treatment.

Factor	First cycle	Second cycle
	Range	Range
pH	3.6–5.6	4.5–5.5
NaCl (mol/*l*)	0–1	0.1–0.4
Pressure (MPa)	50–200	120–250
Temperature (°C)	0–70	15–40
Treatment time (min)	0–100	50–100

The left side is the range for cycle 1 of RCO. The right side is for cycle 2 shrunken down by the result of cycle 1.

Materials and Methods

Materials Hen egg-white lysozyme (Mw. 14,300) was purchased from Sigma Chemical Co. (St. Louis, MO.) and dextran T-10 (Mw. 9500) was from Pharmacia Biotech AB (Uppsala, Sweden). All other chemical reagents used were of the highest grade available.

Optimization of the conditions for preparation of lysozyme-dextran complex The RCO program was obtained directly from the Department of Food Science, the University of British Columbia (Vancouver, BC. Canada) through Dr. Shuryo Nakai. RCO was constructed from multiple cycles and the optimum factor levels found through step-up cycles (Fig. 1). Factors levels of the pH of the 0.1 mol acetate buffer as the solvent for the samples, the concentration of NaCl (mol/*l*) added to the solvent, pressure (MPa), temperature (°C) and treatment time (min) were optimized. The range of each factor is listed in Table 1. The unpredictable changes in the treatment temperature that occurred during the high pressure treatment were corrected at the response input step in the RCO program. The response of each condition is shown as the absorbance at 280 nm for the collected LDC solution. The completion of the maximization was regarded when no other condition had exceeded the previous cycle in response.

Preparation of the lysozyme-dextran complex by high pressure treatment The preparation of LDC in the liquid state using a high pressure treatment was based on a previous study (Nakamura *et al*., 1997). Hen egg-white lysozyme and dextran were mixed in a weight ratio of 1 : 5 and dissolved into 0.1 M acetate buffer containing NaCl. The high pressure treatment of this sample solution was carried out using a high pressure generator equipped with a thermal controlling system (IHI-ITP type 70 ; Ishikawazimaharima Heavy Industry Co., Ltd., Tokyo) and then immediately frozen using dry ice for 60 min. LDC was separated from the high pressure treated resulting solution by gel permeation chromatography using a Sephacryl S-300 packed column (2.5×75 cm, Pharmacia LKB, Uppsala, Sweden). The chromatograph column was equilibrated and eluted with 0.1 M acetate buffer (pH 5.0) containing 50 mM NaCl. The first peak containing protein and carbohydrate were collected as LDC (Nakamura *et al*., 1997). The collected solutions were dialyzed against ultrapure water (PURIC Model-S; Oregano Co., Ltd. Tokyo), concentrated to 50 ml by polyethylene glycol (Mw. 20,000) and then stored at 4°C.

Table 2. Summary data for random-centroid optimization.

Vertex No.	pH	NaCl (M)	Pressure (MPa)	Temp. (°C)	Time (min)	Response (Abs.)	SD (n=3)
0	5.0	0.5	150	60	60	0.397	—
First cycle							
Random search							
1	5.0	0.9	97.07	18.1	81	0.656	0.015
2	**4.5**	**0.1**	**192.14**	**19.3**	**88**	**1.125**	**0.018**
3	4.0	0.3	109.82	23.3	53	0.890	0.001
4	4.0	0.7	189.93	26.9	40	0.535	0.009
5	4.5	0.9	105.02	23.1	35	0.196	0.003
6	4.5	0.3	154.69	36.8	14	0.753	0.024
7	5.5	0.9	86.40	34.4	83	0.142	0.002
8	4.0	0.7	66.57	34.8	73	0.913	0.014
9	5.0	0.4	67.17	54.2	39	0.163	0.004
10	5.5	0.1	169.34	46.4	13	0.316	0.010
Centroid search							
11	4.4	0.54	139.96	31.3	59	0.540	0.001
(*12*	*4.2*	*0.46*	*124.06*	*26.5*	*62*	—	—
(*13*	*4.3*	*0.42*	*142.63*	*28.2*	*54*	—	—
(*14*	*4.4*	*0.54*	*131.11*	*24.5*	*67*	—	—
Second cycle							
Random search							
12	5.0	0.29	148.24	32.8	74	0.912	0.010
13	4.9	0.27	185.21	20.8	91	1.055	0.027
14	4.7	0.40	238.84	31.1	77	0.561	0.010
15	5.4	0.23	242.48	37.7	86	0.978	0.029
16	5.2	0.37	136.08	16.8	56	0.974	0.011
17	5.3	0.10	160.74	33.2	81	0.880	0.033
18	4.6	0.38	147.72	24.9	92	0.654	0.016
19	4.8	0.15	221.31	29.0	86	0.463	0.013
Centroid search							
20	4.8	0.33	164.50	25.9	79	1.102	0.002
(*21*	*5.0*	*0.25*	*180.83*	*25.5*	*79*	—	—
(*22*	*4.8*	*0.32*	*166.93*	*29.0*	*82*	—	—
(*23*	*4.7*	*0.35*	*145.65*	*24.9*	*76*	—	—

The response of each condition was the absorbance at 280 nm of the collected and concentrated LDC solution. Experiments have not been conducted on the conditions of Vertex 12·14 and 21–23 because the responses of Vertex 11 and 20 did not exceed that of Vertex No. 2.

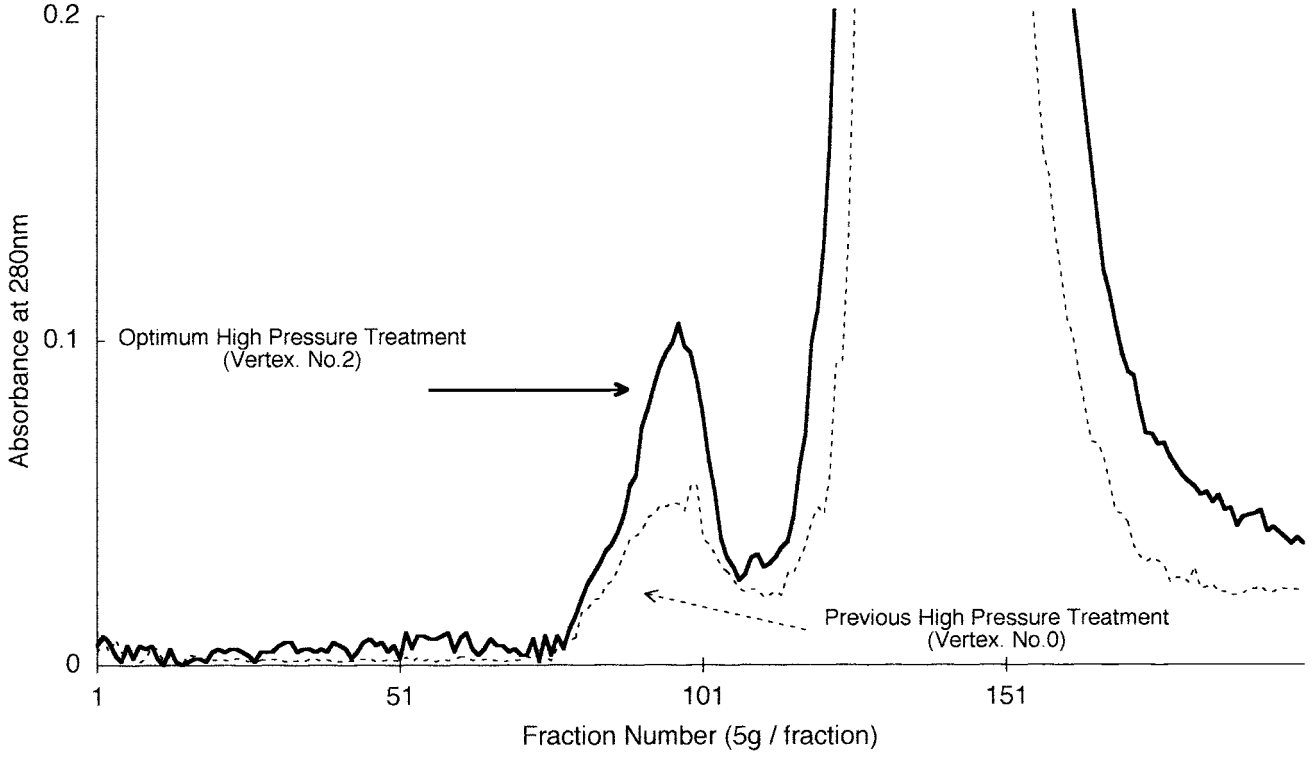

Fig. 2. Elution profiles in 0.1 M acetate buffer (pH 5.0) containing 50 mM sodium chloride on Sephacryl S-300 column on the solutions of lysozyme-dextran complex prepared in liquid state by the high pressure treatment.

Estimation of the amounts of LDC The amounts of LDC were calculated from the absorbances at 280 nm as the protein concentrations of LDC.

Results and Discussion

Optimization for the preparation of LDC in the liquid state using high pressure treatment was accomplished in 20 experiments. The results are summarized in Table 2. The optimum treatment was found as Vertex No.2, because no other condition exceeded its response. In the elution profiles shown in Fig. 2, the production rate of LDC was recognized as the area of the first peak. This optimum treatment increased the production rate of LDC compared with the treatment in the previous study (Vertex No. 0) (Nakamura *et al.*, 1997). The amount of LDC was computed from the absorbance at 280 nm in the collected solution. One tenth of the lysozyme formed the complex with dextran under this condition.

Figure 3 shows the graphical results as maps of optimization. The optimum area was judged from the trend lines on the maps. A trend line has drawn on the map connecting the data points that qualified to be linked. Generally, most trend lines were pointing towards the optimum area from each data point and the more important lines for the maximization were drawn in the upper area of the map. Several trend lines were drawn and indicated certain areas on the map for each factor, thus these five factors interacted and were essential for the preparation of LDC by the high pressure treatment. Perez-Mateos and Montero (1997) reported the interaction among 3 factors, such that pressure, time and temperature were essential for the preparation of the high-pressure induced gel of sardine (*Sardina pilchardus*). In addition to these 3 factors, the pH and the concentration of electrolytes such as NaCl in the solvent were important factors for the protein modification. The optimum condition for high pressure treatment for the preparation of LDC was pH 4.5, 0.1 M NaCl, 192 MPa, 19.3°C and the treatment time of 88 min. The irreversible denaturation of the protein occurred at more than 200 MPa (Balny & Masson, 1993). However, the formation of LDC occurred in a lower pressure range in this study, this indicated that the other four factors contributed to the denaturation of the proteins during the high pressure treatment. Especially, the heat treatment was considered to be significant importance (Nakamura *et al.*, 1997).

Probably, most global optimization methods are more mathematically precise and less practical than RCO, particularly in biological experiments. The computational optimization by the RCO method must be powerful, although it is not a computational optimization in the strict sense because human judgment in mapping is an essential step. For instance, the minimization of the 4-factor Wood's function (Reklaitis *et al.*, 1983) was completed in 49 experiments using the RCO method (Nakai *et al.*, 1998) though 50824 experiments were needed using the level set global optimization (Yassen, 1993). The additional experiments were required to find a more accurate optimum condition, however, further use of the RCO method was not efficient. The RCO program showed the optimum areas to economize time and cost for the examinations compared to the mathematical optimization methods and extreme accuracy was unimportant in the biological research fields (Nakai *et al.*, 1999). The more precise optimum condition might be located in the vicinities of the optimum point found by RCO method by using other techniques such as response surface or conventional simplex search. It is expected that broader applications of RCO should not be limited only to optimizing research and development. A variety of optimization ob-

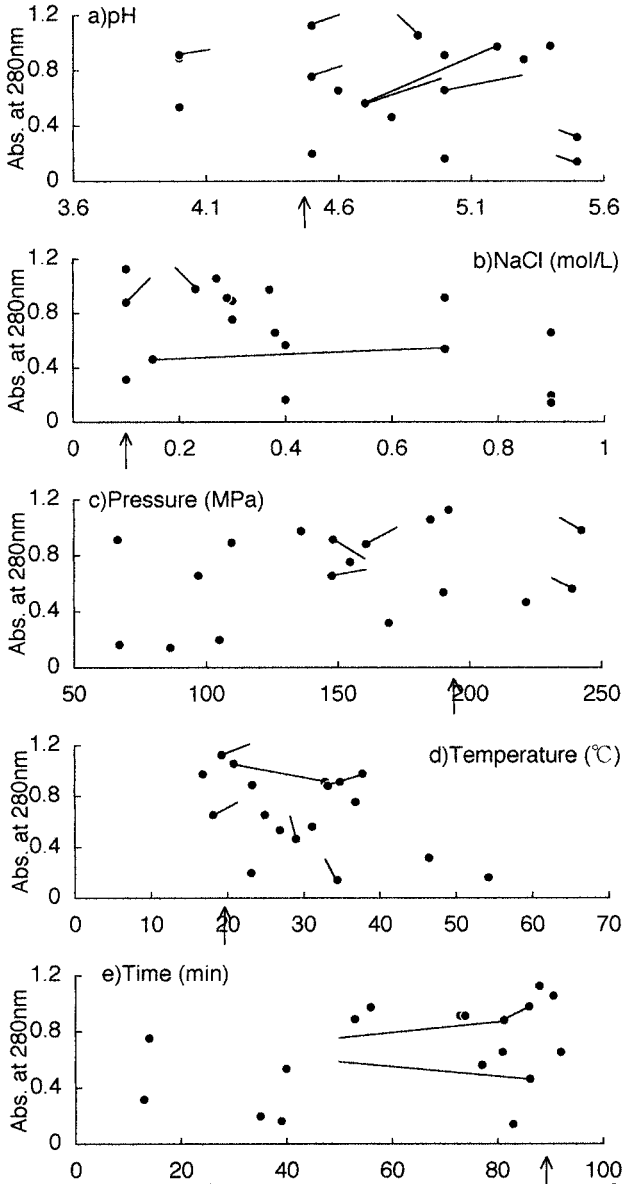

Fig. 3. Graphical results of the maximization of the production rate of lysozyme-dextran complex prepared in the liquid state using high pressure treatment.

jectives in different fields of study can use this optimization method, for instance, to find the best amplifying condition for DNA using PCR (Fukuda, 2000).

Acknowledgments The authors are grateful to Dr. Shuryo Nakai for introduction and application of the RCO program, to Dr. Toshiro Suzuki for applying the high pressure treatment, and to many others for their great support and advice during this study.

References

Balny, C. and Masson, P. (1993). Efects of high pressure on proteins. *Food Rev. Int.,* **9**, 611–628.

Fukuda, Y. (2000). Polymorphism of the Japanese quail of MHC class 1 gene. M. Ag. Thesis, Tokyo University of Agriculture (in Japanese with English Abstract).

Kobayashi, Y., Nakai, S., Horimoto, Y. and Chiji, H. (1997). Optimum composition of ingredients for high-fiber bread containing wheat bran by random centroid optimization. *Bull. Fuji Women's Coll.,* **35**. series II, 31–37.

Nakai, S. (1990). Computer-aided optimization with potential application in biorheology. *J. Jpn. Soc. Biorheol*., **4**, 143–152.

Nakai, S., Dou, J., Lo, K.V. and Scaman, C.H. (1998). Optimization of site-directed mutagenesis. 1. New random-centroid optimization program for Windows useful in research and development. *J. Agric. Food Chem.,* **46**, 1642–1654.

Nakai, S., Saeki, H. and Nakamura, K. (1999). A graphical solution of multimodal optimization to improve food properties. *Int. J. Food Prop.,* **2**, 277–294.

Nakamura, K. (1996). Studies on bactericidal effects of lysozyme-saccharide complexes. M. Ag. Thesis, Tokyo University of Agriculture (in Japanese with English Abstract).

Nakamura, K., Furukawa, N., Matsuoka, A., Takahashi, T. and Yamanaka, Y. (1997). Enzyme activity of lysozyme-dextran complex prepared by high pressure treatment. *Food Sci. Technol. Int. Tokyo.,* **3**, 235–238.

Nakamura, S., Kato, A. and Kobayashi, K. (1990). Novel bifunctional lysozyme-dextran conjugate that acts on both gram-negative and gram-positive bacteria. *Agric. Biol. Chem.,* **54**, 3057–3059.

Nakamura, S., Kato, A. and Kobayashi, K. (1991). New antimicrobial characteristics of lysoyme-dextran conjugate. *J. Agric. Food Chem.,* **39**, 647–650.

Nishimura, K., Goto, M., Imazuya, N. and Nakai, S. (1997). Optimum cooking conditions for indica type rice by using random centroid optimization. *J. Cookery Sci. Jpn.,* **30**, 9–16. (in Japanese with English Abstract)

Nishimura, K., Imazuya, N. and Nakai, S. (1998). Optimum preparative method for storing cream puff paste without deterioration. *Food Sci. Technol. Int. Tokyo.,* **4**, 18–24.

Perez-Mateos, M. and Montero, P. (1997). High-pressure-induced gel of sardine (*Sardina pilchardus*) washed mince as affected by pressure-time-temperarure. *J. Food Sci.,* **62**, 1183–1188.

Reklaitis, B.V., Ravindram, A. and Ragasdell, K.M. (1983). "Engineering Optimization: Methods of Application." ed. by Wiley-Interscience Publication, New York

Tamaoka, T., Itoh, N. and Hayashi, R. (1991). High pressure effect on maillard reaction. *Agric. Biol. Chem.,* **55**, 2071–2074.

Visweswaran, V. and Floudas, C.A. (1990). A global optimization algorithm (GOP) for certain class of non convex NLPsII. Appication of theory and test problems. *Comput. Chem. Eng.,* **14**, 1419–1434.

Yassen, H.A. (1993). A level set global optimization method for nonlinear engineering problems. Ph.D. Thesis. University of British Columbia.