Food Chemistry 227 (2017) 264–270

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Artificial neural network – Genetic algorithm to optimize wheat germ fermentation condition: Application to the production of two anti-tumor benzoquinones

Zi-Yi Zheng, Xiao-Na Guo, Ke-Xue Zhu *, Wei Peng, Hui-Ming Zhou *

State Key Laboratory of Food Science and Technology, Collaborative Innovation Center for Food Safety and Quality Control, School of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi 214122, Jiangsu Province, People's Republic of China

ARTICLE INFO

Article history:
Received 20 June 2016
Received in revised form 23 December 2016
Accepted 15 January 2017
Available online 18 January 2017

Keywords:
Wheat germ
Benzoquinones
Fermentation
Artificial neural network
Second-order interactions

ABSTRACT

Methoxy-ρ-benzoquinone (MBQ) and 2, 6-dimethoxy-ρ-benzoquinone (DMBQ) are two potential anti-cancer compounds in fermented wheat germ. In present study, modeling and optimization of added macronutrients, microelements, vitamins for producing MBQ and DMBQ was investigated using artificial neural network (ANN) combined with genetic algorithm (GA). A configuration of 16-11-1 ANN model with Levenberg-Marquardt training algorithm was applied for modeling the complicated nonlinear interactions among 16 nutrients in fermentation process. Under the guidance of optimized scheme, the total contents of MBQ and DMBQ was improved by 117% compared with that in the control group. Further, by evaluating the relative importance of each nutrient in terms of the two benzoquinones' yield, macronutrients and microelements were found to have a greater influence than most of vitamins. It was also observed that a number of interactions between nutrients affected the yield of MBQ and DMBQ remarkably.

1. Introduction

Wheat germ, which is the by-product of flour-milling industry, contains various bioactive compounds which are beneficial to human physiology (Rizzello, Cassone, Coda, & Gobbetti, 2011). In the past few decades, several studies demonstrated the safety of fermented wheat germ extract for its intended use as a dietary supplement ingredient (Roberta et al., 2002). According to the findings of literatures, fermented (by *Saccharomyces cerevisiae*) wheat germ extract exhibited cytotoxic activity towards cancer cell lines and positive immunological effects (Jakab et al., 2003). It is currently assumed that two methoxy-substituted benzoquinones, i.e., methoxy-ρ-benzoquinone (MBQ) and 2,6-dimethoxy-ρ-benzoquinone (DMBQ) were responsible for the biological properties (Mueller, Jordan, & Voigt, 2011). The two benzoquinones were derived from hydroquinones substituted by β-1,6-linked oligosaccharides in wheat germ (Zhokhov, Broberg, Kenne, & Jastrebova, 2010). In the fermentation process, β-glucosidic linkages of hydroquinones glucosides were split by β-glucosidase (EC 3.2.1.21) of *Saccharomyces cerevisiae* to form methoxy-ρ-hydroquinone and 2, 6-dimethoxy-ρ-hydroquinone, as shown in Fig. S1. Further, the two hydroquinones were catalyzed by wheat germ peroxidase (WGP) to form MBQ and DMBQ (Garcia, Rakotozafy, Telef, Potus, & Nicolas, 2002). From the above description, the production of high-price bioactives (MBQ and DMBQ) from fermented wheat germ requires high activities of β-glucosidase and peroxidase. However, the activities of β-glucosidase and peroxidase in fermentation broth were low and unsatisfactory for the bioconversion of the hydroquinone glucosides (Rizzello et al., 2013).

Exogenous β-glucosidase and peroxidase could be added into fermentation broth to enhance the yields of MBQ and DMBQ. However, high costs and loss of activities during the fermentation process are two major obstacles of exogenous enzymes. It is well known that nutrients, i.e. macronutrients, microelements and vitamins are essential for microbial metabolism. Further, some macronutrients and microelements have been proven to modulate enzyme activities due to their location in the active site of enzyme (Jeng et al., 2011). Therefore, addition of appropriate amount of nutrients might be speculated to increase the yields of MBQ and DMBQ.

There is a crucial need to find a predictive model to illuminate the complicated law between multiple nutrients and two benzoquinones in wheat germ fermentation process. Traditional modeling and optimization approaches for multiple variables such as response surface methodology, present restrictions for modeling highly complex biological systems (Rafigh, Yazdi, Vossoughi,

* Corresponding authors.
E-mail addresses: 18206188734@163.com (Z.-Y. Zheng), xiaonaguo@jiangnan.edu.cn (X.-N. Guo), kxzhu@jiangnan.edu.cn (K.-X. Zhu), pengwei@jiangnan.edu.cn (W. Peng), hmzhou@jiangnan.edu.cn (H.-M. Zhou).

http://dx.doi.org/10.1016/j.foodchem.2017.01.077
0308-8146/

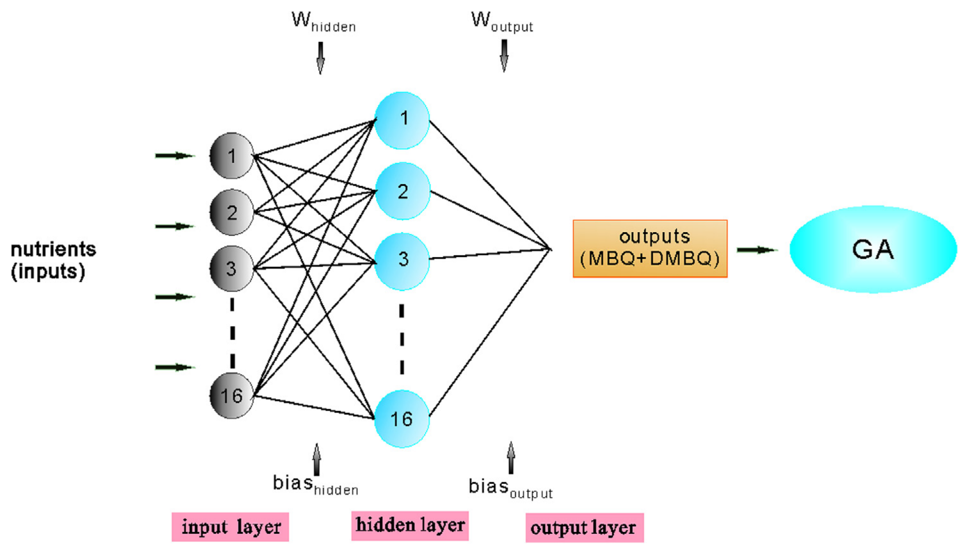

Fig. 1. Schematic diagram of the combined back-propagation artificial neural network modeling and genetic algorithm. The connections between nodes are associated with weights and biases.

Safekordi, & Ardjmand, 2014). In the last decade, artificial neural network (ANN), which exhibited high accuracy and generalization ability in modeling, has been widely applied to model the non-linear biological systems (Cimpoiu, Cristea, Hosu, Sandru, & Seserman, 2011; Hosu, Cristea, & Cimpoiu, 2014; Vani, Sukumaran, & Savithri, 2015). A common artificial neural network is a connected parallel architecture consisting of an input layer composed of neurons (corresponding to the input variables), a hidden layers composed of neurons and an output layer composed of neurons (corresponding to the output variables), as schematized in Fig. 1. Genetic algorithm (GA) is a stochastic global optimizing algorithm, which is based on the laws of biological evolution (Kumar, Pathak, & Guria, 2015). GA has been shown the ability to solve smooth or non-smooth optimization and this algorithm does not require differentiable or continuous functions. Applying of GA has been proved to be effective in improving the productivity of fermentation (Camacho-Rodríguez, Cerón-García, Fernández-Sevilla, & Molina-Grima, 2015; García-Camacho, Gallardo-Rodríguez, Sánchez-Mirón, Chisti, & Molina-Grima, 2011; Kumar et al., 2015).

The fermentation parameters, i.e. agitation speed, initial pH, fermentation temperature and fermentation time have been optimized in order to maximize the total contents of MBQ and DMBQ in our previous study (Zheng, Guo, Zhu, Peng, & Zhou, 2016). In present work, ANN was assessed as a predictive model between the total contents of MBQ and DMBQ in fermented wheat germ and 16 nutrients (macronutrients, micronutrients and vitamins). Then two approaches of evaluating the relative importance of each nutrient for the total contents of MBQ and DMBQ was performed. Moreover, to evaluate nutrients' second order interactions for the total contents of MBQ and DMBQ, a novel method was established based on sixteen newly established ANN models with partial derivatives as the outputs. Finally, an optimal formulation of exogenous nutrients to achieve the maximum total contents of MBQ and DMBQ was worked out by the combined ANN-GA method.

2. Materials and methods

2.1. Materials

The raw wheat germ was obtained from Yihai Kerry Food Industry Co, Ltd (Kunshan, China). It was stored at −18 °C in a freezer before experiments. Calcium chloride ($CaCl_2$), Magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$), iron vitriol, ($FeSO_4{\cdot}7H_2O$), copper sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$), zinc sulfate heptahydrate ($ZnSO_4{\cdot}7H_2O$), Manganese sulfate monohydrate ($MnSO_4{\cdot}H_2O$), selenium (Se), thiamine hydrochloride (B_1), riboflavin (B_2), nicotinic acid (B_3), calcium d-pantothenate (B_5), pyridoxine hydrochloride (B_6), Cyanocobalamin (B_{12}), 2-methyl-1,4-naphthoquinone (K_3), 1-naphthalenol, 4-amino-2-methyl-,hydrochloride (1:1) (K_5), and d-ascorbic acid (C) were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Methoxy-ρ-benzoquinone, and 2,6-ρ-dimethoxybenzoquinone were purchased from Meilian biotechnology Co., Ltd (Shanghai, China).

2.2. Media preparation and fermentation

The strain *S. cerevisiae* (RC212, Huankai Biological Technology Co., Ltd, Guang Zhou, China) were cultivated on a YPD (1% yeast extract, 1% peptone, 2% glucose, 2% agar) medium in a 500 ml Erlenmeyer flask under aerobic condition with chloramphenicol (0.01%) to avoid bacterial growth. The Erlenmeyer flask was incubated in a controlled incubator shaking at 30 °C. Then *S. cerevisiae* was transferred into liquid YPD overnight under aerobic condition with constant shaking at 30 °C in Erlenmeyer flasks. For all experiments, *S. cerevisiae* was collected at the beginning of the exponential growth phase (OD_{600nm} = 1.1). 5 ml *S. cerevisiae* suspension was inoculated in a 500 ml Erlenmeyer flask with 200 ml tap water and 10 g wheat germ.

Sixteen nutrients including macronutrients (Ca, Mg), micronutrients (Fe, Cu, Zn, Mn, Se), vitamins (B_1, B_2, B_3, B_5, B_6, B_{12}, K_3, K_5, C) were added into medium before fermentation. The concentration range of each component was determined by single – factor experiments and could be seen in Supplementary Figs. S4–S19. Erlenmeyer flask was held on an orbital shaker incubator at 142 rpm, 31.6 °C. After 39.8 h of fermentation, each sample was freeze-dried and then kept in the refrigerator. The agitation speed, fermentation temperature and time were optimized previously.

2.3. Determination of MBQ and DMBQ

Ten grams of lyophilized sample were dissolved in 250 ml of double distilled water and extracted three times by shaking with 100 ml $CHCl_3$. $CHCl_3$ layers were collected and washed three times