LWT - Food Science and Technology 87 (2018) 259–265

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Development of new red mold rice and determination of their properties

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ARTICLE INFO

Article history:
Received 21 March 2017
Received in revised form
21 August 2017
Accepted 24 August 2017
Available online 4 September 2017

Keywords:
RMR
Herbs
Enzyme activity
Rice wine
Antioxidant capacity

ABSTRACT

In order to analyze the effect of Chinese herbs on the three major enzyme activities: glucoamylase, protease and esterase as well as the biomass of the *Monascus* spp., a high quality Red mold rice (RMR) with Chinese herbs was developed. The new RMR demonstrated higher enzyme activity and biomass than the blank control. The glucoamylase, protease and esterase activity were increased to 518.03 U/g, 10.10 g/kg and 30.99 g/kg, respectively. Simultaneously, the biomass increased to 59.82 g/kg. With its application in rice wine, the alcohol yield ratio, total amino acid and the fragrance composition content increased obviously, respectively, and the organic acid content and the antioxidant capacity in vitro increased markedly.

1. Introduction

Red mold rice (RMR) is prepared by *Monascus* spp. growing in solid-state culture. It has been used in alcoholic drinks and natural edible pigment for many centuries, as well as a traditional medicine for hypolipidemic and hypotensive effects (Lee & Pan, 2011). As the fungi *Monascus* spp. synthesis multiple functional secondary metabolites, mainly containing γ-Aminobutyric acid (GABA) and monacolin K (MK), RMR showed an important medical value in prevention of diseases such as hypercholesterolemia, hypertension and Alzheimer's disease (Shi & Pan, 2012). As it reported, GABA is an antihypertension drug, and MK is an effective cholesterol-lowering drug in inhibiting the 3-hydroxy-3-methylglutaryl-coenzymeA reductase (HMG-CoA), a rate-limiting enzyme in regulation of cholesterol biosynthesis (Lee, Hung, Wang, & Pan, 2007; Su, Wang, Lin, & Pan, 2003).

Chinese traditional *Yaoqu* is a fermented production by several kinds of microorganisms, mainly consisting of *Rhizopus* spp., *Aspergillus* spp. and yeasts, which grow in mixed solid-state culture with raw starchy grains and several herbs. *Yaoqu* contributes to supply abundant enzymes and synthesis special flavor and functional components during fermentation (Li et al., 2014; Zhang, Wu, Zhang, Wang, & Li, 2009). In order to develop a new *Yaoqu* with high and stable quality, the *Panax quinquefolius Yaoqu* with high enzyme activities and functional components was applied, which was fermented by the fungi *Aspergillus oryzae* QJ and the herb *Panax quinquefolius* (Li et al., 2014; Yang, 2005).

There are multiple enzymes involving in each biochemical reaction in microorganism, the enzymes impact the microbial growth and metabolism significantly, and relevant to the primary metabolites though supplying basic nutrients for growth of *Monascus* spp … In this research, the new red mold rice with *Panax quinquefolius*, *Rhodiola rosea* and *Paeonia lactiflora* were developed separately and first applied to the rice wine in our laboratory. Furthermore, the glucoamylase activity, protease activity, esterase activity and biomass in new red mold rice; besides the detection of ehanol concentration, alcohol yield, pH, total acid, amino nitrogen and sugar contents in rice wine were carried out. The new red mold rice, a special starter culture, plays a key role in liquor brewing. It's not only contributes to starch degradation and play a role enzyme activities during fermentation, but also determines the flavor and special healthcare function.

There are few reports about the influence of medium

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http://dx.doi.org/10.1016/j.lwt.2017.08.088
0023-6438/

components on these enzymes of *Monascus* spp., especially the effect of varied herbs. This study focuses on the effects of herbs on the enzymes and biomass during the RMR processing. It is the first report showing that these herbs, including *Panax quinquefolius, Rhodiola rosea* and *Paeonia lactiflora,* have a significant promotion on the three major enzymes metabolism of *Monascus* spp..

2. Materials and methods

2.1. Materials

2.1.1. Microorganisms and herbs

The strains include *Monascus rubber* QH from Qianhe Condiment Co., Ltd (Meishan, China), the yeasts and *Saccharomyces cerevisiae* purchased from Angel Yeast Co., Ltd. (Yichang, Hubei, China). These Chinese herbs, *Panax quinquefolius, Rhodiola rosea* and *Paeonia lactiflora,* were purchased from a local pharmacy (Chengdu, China).

2.1.2. Seed culture

The *Monascus rubber* QH spore was cultured in rice-koji extract for incubation at 28 °C for 4 days.

2.2. Preprocessing method

2.2.1. Preparation of solid-state medium with or without herbs

The rice was prepared after soaking for 45–60 min, steaming for 30–45 min, and cooling down to 30°C-35 °C with the retained moisture content at 30–35% (Lee & Pan, 2012). The herbs were preprocessed into two types: powder and water extract of herbs. Mixed with or without single herbs into the steamed rice, the solid-state medium was prepared for RMR fermentation.

2.2.2. Fermentation of RMR

Herbs were firstly crushed to powder by grinder, and impurities were eliminated by sieve. The rice was cleaned three times and soaked in water for 45 min, and then the water was drained for half an hour. The rice was further steamed for 45 min and cooled down to 30 °C (Zhao et al., 2012). Then, the QH spore bacteria suspension (1.0×10^5 spores per gram of rice) was added to the steamed rice (the moisture content of 30–35%) under aseptic conditions, and single herb powder (6.0 g/kg of the mass of steamed rice) was added to the rice. The mixture of rice, spore, and herbs powder was incubated in a cultivation cabinet with constant temperature and humidity. The cultivating condition is described as follows: temperature 33–35 °C, 95–98% humidity for 4–6 days, then temperature 32 °C, humidity 95% for 1–2 days, and finally drying training 40 °C for 12 h.

2.2.3. Fermentation of red rice wine

Baseed on Hao's report (Hao et al., 2011), the three times fed-batch fermentation was used in red rice wine brewing in which the mixture addition of steamed rice as substrate, the new red mold rice with herbs as saccharifying agents and alcohol active instant dried yeasts were fermenting agents. The results shown in Fig. 1, a blank control was not added the herbs in red mold rice.

2.3. Analytical methods

2.3.1. Determination of enzymes activities and biomass of M. rubber QH

The glucoamylase, protease and esterase activity, were measured according to the literature method with modification (Xu et al., 2001). The determination of biomass was based on the report with modification (Liu, Xu, & Cen, 2000).

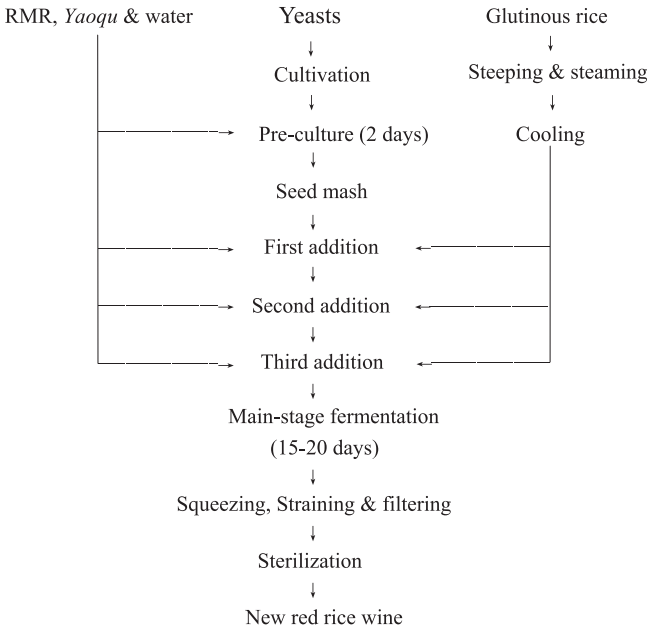

Fig. 1. Fed-batch fermentation process of red rice wine.
The pre-culture was carried out in 5 L jar fermentorfor cultivation at 28 °C for 2 days. The main-stage fermentation was carried out by the next three batches of the mixture addition in the same fermentor below 30 °C for 15–20 days. Then, all the fermented mash was reprocessed as following: squeezing, straining, filtration through 0.50 μm microporous filter membrane, sterilization at 80 °C for 30 min and cryogenic storage below 15 °C.

Glucoamylase activity. About 90 mL water and 10 mL buffer (sodium acetate-acetic acid buffer, 2 mol/L, pH = 4.6) were added to 5 g dried RMR, and the extracted solution was in water bath at 30 °C for 1 h with stirring every 15 min. The extracts were further filtered and received about 50 mL. Secondly, 50 mL 2% soluble starch solution was added in 100mLvolumetric flask, bathed at 35 °C for 20 min with the addition of accurate 10 mL enzyme extracts, and the starch and enzyme reacted in water bath at 35 °C for 1 h. About 3 mL sodium hydroxide (1 mol/L) was used to stop the reaction, which was diluted to 100 mL with distilled water at room temperature, and the solution was alkaline. To prepare the blank test solution, 3 mL sodium hydroxide (1 mol/L) was added at first and the other process was the same as the saccharifying test solution of RMR extracts. Thirdly, the saccharifying test solution of RMR extracts was determined by Fehling reagent titration. About 5 mL Fehling A and B, 10 mL distilled water, and appropriate amount of the 0.1% standard glucose solution were added in RMR extracts that was accurated to 1 mL, the mixture solution was heated to boiling on a hot plate, and titrated with the 0.1% standard glucose solution to disappearance of the blue within 1 min. The blank test solution was substituted for the saccharifying test solution of RMR extracts, and the other process was the same as the determination of the saccharifying test solution of RMR extracts. All the tests were performed in triplicate and the results averaged.

$$\text{The glucoamylase activity(ga)} = \frac{(v_0 - v) \times \rho}{5 \times \frac{10}{100} \times \frac{5}{100}} \times 100$$

v_0 = the volume of 0.1% standard sugar consumed during the determination of the blank test solution (mL)
v = the volume of 0.1% standard sugar consumed during the determination of the saccharifying test solution of *Yaoqu* extracts (mL)