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Development of the dietary fiber functional food and studies on its toxicological and physiologic properties

Yan Hong [a,b,*], Wang Zi-jun [a,b], Xiong Jian [a,b], Dai Ying-jie [c], Ma Fang [d,*]

[a] College of Chemical and Environmental Engineering, Harbin University of Science and Technology, Harbin 150040, China
[b] Key Laboratory of Green Chemical Technology of College of Heilongjiang Province, Harbin 150040, China
[c] School of Resources Environment, Northeast Agricultural University, Harbin 150030, PR China
[d] State Key Laboratory of Urban Water Resource and Environment, Harbin Institute of Technology, Harbin 150090, China

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ABSTRACT

Dietary fiber (DF) obtained from wheat bran by microbial fermentation was used as a food additive to cookies. The cookies were evaluated sensorally through an orthogonal test to gain the optimized production conditions as follows: the suitable DF content 8%, leavening agent 1.5%, standing time 5 min, and baking time of the cookies is 8 min.

A series of toxicological and physiological functions of the cookies were studied using KM mice as the experimental animal in this paper. No deaths or abnormal behaviors of mice occurred either in acute toxicity tests or in short-term feeding tests. Besides, the weight gains, food utilization ratios, blood and serum biochemical parameters, organ coefficients and the results of organ histopathology tests of all doses groups exhibited no significant differences with the control group. This reveals that the dietary fiber functional cookies made by this formula have no acute or sub-chronic toxicity. In terms of physiological function, compared with the control group, the total cholesterol (TC) and triglycerides (TG) were 17.0–21.7% and 18.7–35.0% lower in mice serum of all DF cookie doses groups, respectively, but this difference was not significant ($P > 0.05$). Compared with positive control group, the Cd^{2+} and Pb^{2+} excretion ratios of DF group were 27.4% and 25.2% higher, respectively. Thus, a conclusion has been drawn that dietary fiber functional cookies made by this formula have no toxic or harmful actions on animals or humans, and the DF food was able to decrease TC and TG concentrations to some extent in serum and increase excretion of Cd^{2+} and Pb^{2+} in Feces.

1. Introduction

As described by American Association of Cereal Chemists (AACC) (2001), dietary fiber is the remnant of the edible part of plants and analogous carbohydrates that are resistant to digestion and absorption in the human small intestine, with complete or partial fermentation in the human large intestine. It includes polysaccharides, oligosaccharides, lignin and associated plant substances. Dietary fiber exhibits one or more of laxation (faecal bulking and softening, increased frequency and/or regularity), blood cholesterol, and/or blood glucose attenuation.

Nowadays, there is a growing demand for a new generation of healthier food products which at the same time have excellent sensory qualities (Ang, 2001; Wang et al., 2008). Consequently, dietary fiber functional food has been paid more attention because of its good performance in physiological function and appropriate taste, etc.

DF functional food can help prevent several diseases. There are chemical side chain groups in the structure of dietary fiber (Baghurst et al., 1996; MeDougall et. al, 1996), such as carboxyl and hydroxyl, which act as the cation exchange resin. Various studies (Hosig et al., 1996; Schneeman, 1986; Truswell, 1995; Lu et al., 2007; Yang et al., 2007) have shown that many cations can be exchanged by DF, especially some toxic cations. Then, such absorbed toxic ions can be excreted with the feces. DF can also absorb some of the harmful substances in animals (Pandolf and Clydesdale, 1992; Yan et al., 2011), which play a role in disease prevention. For example, it has been proven that adsorption of NO^{2-} and bile acid can prevent cancer, high blood pressure (Behall et al., 2006), heart disease (Bourdon et al., 2001; Fernandez, 1995) and cardiovascular disease (Anderson and Tietyen-Clark, 1986; Merchant et al., 2003). Moreover, it can help reduce the rate of glucose absorption (Katriina et al., 2003; Chandalia et al., 2000), constipation (Goodland, 2007; Zhong et al., 2004), the levels of total cholesterol (Brown et al., 1999; Nishina and Freedland, 1990; Tucker and Thomas, 2009) and triglycerides, inhibit the synthesis of hepatic fatty acid and maintain stable blood sugar levels after meals which

* Corresponding author.
E-mail address: mafang@hit.edu.cn (M. Fang).

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is very important for diabetics (Wolever et al., 2004; Vuksan et al., 2000; Brownlee, 2009).

Simultaneously, appropriate DF does not reduce the taste of food, but adds some new flavor and improve the quality. As textural studies revealed (Gomez et al., 2003), DF additions, in general, had apparent effects on dough properties yielding higher water absorption, mixing tolerance and tenacity, and smaller extensibility in comparison with those obtained without fiber addition. Besides, the fiber always leads to a longer shelf life. The main problem of DF addition in baking is the important reduction of loaf volume and the different texture of the breads obtained (Guillon and Champ, 2000). So, the optimal proportion of DF adding in each different food should be researched and determined in its development process.

Generally, there are three methods to gain dietary fiber: Chemical method, physical method and microbial fermentation.

Removal of starch and protein can be more complete using chemical method, but the poor selectivity, side-effects and difficultly controlled extraction conditions greatly limit its use (Wang et al., 2004; Du et al., 2005). What are worse, hemicelluloses and soluble dietary fiber (SDF) which plays an important role in physiological function is soluble in alkaline solution. Thus, this method can cause the undesired decrease of overall physiological activity (Zhang et al., 2011).

Physical method, such as extrusion cooking, does not cause degradation of the polymer structure or some other deep damage. Therefore, the side chain group can be preserved almost intact, which enables the cation exchange capacity not to be impacted (Ma et al., 2005; Liu et al., 2005; Jacobs and Delcour, 1998).

Recently, microbial fermentation of dietary fiber has been widely recognized and accepted due to the high selectivity, mild and easily controlled reaction conditions (Liu, 2008). It has also the advantages of not destroying the structure of natural fiber and no loss of important physiologically functional SDF and hemicelluloses. However, the microbial fermentation itself is still in its infancy stage (Li, 2003) and microbial fermentation of DF may produce toxic substances, thus affecting its safety.

Therefore, in this study, wheat bran was fermented by the screened efficient lignin-degrading fungi 16-2 to obtain the dietary fiber products with high-quality properties, biological activity and safety. DF obtained from wheat bran was added to cookies as a kind of functional additive. To produce the fiber-rich functional foods with best sensory characteristics, sensory evaluation was used as an indicator to adjust the production process and the proportion of additives. In addition, animal feeding experiments of DF functional food is conducted to explore its toxicological effects and physiological functions.

2. Materials and methods

2.1. Preparation of DF

DF raw materials were agricultural waste (wheat bran) from Heilongjiang Province. The efficient strains used in this study (lignin-degrading fungi 16-2) were isolated in early experiments by our laboratory.

Strains were expanded on potato-dextrose agar plates previously. Two strain plugs with the diameter of 6 mm were inoculated into 250 mL flasks containing 150 mL liquid medium (pH6.0) with 20 g wheat bran as substrate. The liquid medium contained (per liter): KH_2PO_4 6.5 g, $(NH_4)_2SO_4$ 6 g, $MgSO_4$ 2.5 g, $CaCl_2$ 1.5 g. Fermentation were performed at 28 °C and 150 r/min for 72 h.

After the fermentation, crude products underwent ethanol precipitation, pumping filtration, washing off TDF residue with ethanol, drying in an oven at 110 °C, and were finally preserved in flasks at room temperature.

2.2. Development of DF cookies

2.2.1. Manufacture

The baking formula based on flour (refined wheat flour) weight included salt (2%), sugar (15%), soybean oil (2%), dietary fiber and leavening agent. Flour kneaded with water at 40 °C and set aside at room temperature for 5–10 min. After the short time of fermentation, the dough mass was pressed to 4 mm thick, then molded. These raw cookies were transferred to a baking pan, immediately baked at 180 °C for 4–8 min. In order to gain the optimum conditions, the content of various additives and different manufacturing processes were tested through orthogonal experiments and the results are shown in Table 1.

Table 1
Factors and levels obtained from orthogonal experiments.

Level	Factor			
	DF content (%)	Leavening agent content (%)	Standing time (min)	Baking time (min)
1	4	1	5	4
2	6	1.5	10	6
3	8	2	15	8

2.2.2. Sensory evaluation

Based on the principles of sensory evaluation of food (Amerine et al., 1965), the untrained panel, consisting of 12 college students (three females and nine males), most of whom had no experience with sensory evaluation techniques, were given a general orientation session to familiarize them with the test procedures. The group evaluated the cookie samples within 1 h after baking. All testing for the group took place between 16:00 and 17:00 in a food testing room maintained at 22 °C with adequate lighting, no disturbing noises and a continuous circulation of air. Each batch of test samples was presented to each panelist in a separate dish labeled with a three-digit random-number code. Eight kinds of sensory (flatness of biscuits, crispness, viscosity of chewing, hardness, sense of exquisiteness, color, smell and taste) were established with a scoring scale of 1 (unsatisfied) to 5 (satisfied).

2.3. Toxicology study

KM mice, mice food and feeding tools were provided by the Harbin veterinary research institute.

Feed Rodent housing and feeding guidelines conformed to the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and the US Department of Agriculture through the Animal Welfare Act (Public Law 99–198).

2.3.1. Acute toxicity test

A total of 50 KM mice, body weights ranging from 18 to 22 g, were used in this study. The animals were randomly divided into five groups (five males, five females per group) and housed five per cage. According to Horn's Dose Escalation method, DF cookies was administered daily by oral at doses of 1.00, 2.15, 4.64, 10 and 21.5 g/kg. Mice in this study were examined twice daily for mortality and signs of ill health or reaction to chemicals under the guidance of acute toxicity (Dai et al., 1997a,b).

2.3.2. Short-term feeding test

A total of 40 KM mice, body weights ranging from 25 to 35 g, were used in this study. The animals were randomly divided into three dose groups and one control group (five males, five females per group) and housed five per cage. Each cage was equipped with an extra and removable lid which jointed bulkheads to make sure that the mice could only eat food that was assigned to them. Mice in all groups were fed 10% of its body weight food daily and the body weight of mice was measured and recorded once a week to update the food intake. Food composition was as follows: (1) Control group: mice were given a fixed amount of Mice Food (10% of its body weight without DF cookies). (2) Three dose groups: according to the proportion of 2.5, 5.0, 10.0 g/kg, DF cookies were mixed with the Mice Food to form the diet for three dose groups, respectively. The mixed dietary was made through a process of weighing, crushing, mixing, adding water, extruding, and then redrying. These mice were dosed by oral and observed of morphological and physiological activities daily for 30 consecutive days and all of each dose group were necropsied at day 31. Different effects from all groups on the gain of body weight, food utilization ratio, blood and serum biochemical parameters, organ coefficient and organ histopathology were analyzed by F-test to evaluate the sub-chronic toxicity of the DF cookies (Dai et al., 1997a,b; Miao, 2003).

2.4. Physiological function study

2.4.1. Functional assay of reducing blood lipids

This function was assessed by the data of TC and TG between control group and other three dose groups from the short-term feeding experiment, using the ECHO-PLUS automatic biochemistry analyzer. *F*-test was used to evaluate whether there were significant differences between the control group and the dose groups (Yang et al., 1996). In this experiment, all blood was drawn (not fasted) from eye socket under CO_2 anesthesia and mice were euthanized immediately after collecting blood.