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# Immunomodulatory activities of non-prolamin proteins in wheat germ and gluten

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ABSTRACT

Albumin (Alb), globulin (Glo), glutelin (Gll) and glutenin (Gln) were separately extracted from wheat germ and wheat gluten. Amino acisd composition, molecular weight distribution, solubility, in vitro digestibility, and immunomodulatory activities were all analyzed. Gll and Gln have similar molecular weight distributions, which differed from those of Alb and Glo. Alb showed the highest solubility at various pH values (except pH 4.0), whereas Glo showed the highest in vitro digestibility. Glo and Gll have the highest proportion of essential to total amino acids, while Alb and Gll have the highest protein digestibility-corrected amino acid scores. Gll had the strongest immunomodulatory effects in terms of stimulation of RAW 264.7 cells to produce IL-6, TNF-α, and IL-10, and good stimulatory effects on splenocyte proliferation, production of IL-2, phagocytosis, and secretion of nitric oxide in RAW 264.7 cells. Gll can be considered a good protein source for use in health foods.



## 1. Introduction

Dietary proteins have extensive practical applications based on their nutritional, functional and biological characteristics (Bauer et al., 2013). Animal-source proteins (such as meat, milk and eggs) have been used as nutritional supplements for a long time; additionally, some animal-source proteins, such as immunoglobulin, have been used in the clinic for the treatment of patients with immune dysfunction (Sapan et al., 2007). Nevertheless, the risks of obesity, diabetes and cardiovascular disease associated with animal-source foods (Igl et al., 2013) have been increasingly acknowledged. It is difficult to satisfy the daily demand with traditional animal-source proteins; therefore, the search for suitable plant-source proteins has been receiving more and more attention.

Wheat is a common cereal and serves as a staple food worldwide. Wheat kernels contain bran, germ, and endosperm; both wheat germ and endosperm are involved in germination and development (He et al., 2015). Defatted wheat germ (DWG) and wheat gluten are by-products of the production of wheat germ oil (Liu et al., 2013) and starch (Scherf et al., 2016), respectively. The protein contents of DWG and wheat gluten are 30% and 75%, respectively, the bulk of which among DWG proteins (DWGPs) is represented by albumins (Alb), globulins (Glo) and glutelins (Gll), while wheat gluten is composed of almost equal amounts of gliadins and glutenins (Gln) (Liu et al., 2013; Scherf et al., 2016; Zhu et al., 2006). In recent years, both DWGP and wheat gluten have been widely studied. High nitrogen solubility, and emulsifying and have foaming properties resulted in extensive use of DWGPs in the food industry (Brandolini and Hidalgo, 2012). Water- and salt-soluble extracts of DWG have immunological activity, and the water extract also has antitumour and antioxidant properties (Zhu

*Abbreviations:* $A_{412}$, optical density measured at 412 nm; Alb, albumin; C, protein concentration; Con A, concanavalin A; D, dilution coefficient; DMEM, Dulbecco's modified Eagle's medium; DTNB, 5,5′-dithiobis-2,2′-nitrobenzoic acid; DWG, defatted wheat germ; DWGP, defatted wheat germ protein; ELISA, enzyme-linked immunosorbent assay; E/S, ratio of essential to total amino acid; FAO/WHO, Food and Agriculture Organization of the United Nations/World Health Organization; FCS, fetal calf serum; Glo, globulin; Gll, glutelin; Gln, glutenin; HMW-GS, high molecular weight glutenin subuints; HPLC, high performance liquid chromatography; IL-1β, interleukin-1β; IL-2, interleukin-2; IL-4, interleukin-4; IL-6, interleukin-6; IL-10, interleukin-10; CE TAL, chromogenic end-point tachypleus amebocyte lysate; LMW-GS, low molecular weight glutenin subunits; LPS, lipopolysaccharide; MW, molecular weight; N, protein conversion factor; NO, nitric oxide; OD, optical density; $P_{sup}$, protein content in the supernatant; $P_{total}$, total protein content in suspension; PBS, phosphate buffer saline; PDCAAS, protein digestibility-corrected amino acid score; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SH, sulfhydryl; SI, stimulation index; SPI, soy protein isolate; SPSS, statistical package for the social sciences; S-S, disulfide bonds; TCA, trichloroacetic acid; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

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et al., 2011). However, no study has been published identifying an active ingredient of DWG with the above-mentioned physiological activities. As for wheat gluten, the studies have mainly focused on the use of the unique viscoelasticity of gluten to prepare valuable films (Chen et al., 2014), modification of gluten by physical, chemical, and biological methods for application to food additives (Liu and Ma, 2016), and hydrolysis of gluten to obtain bioactive peptides (Cian et al., 2015).

Our study aimed to find an excellent wheat-source protein, with particular focus on the immunological effects of such proteins. Owing to the poor solubility of prolamin and gliadins in aqueous solution, the allergenicity of gliadin (Scherf et al., 2016), and the low concentration of prolamin (Zhu et al., 2006) in wheat germ, only Alb, Glo, Gll, and Gln were characterized in the present study. This study first compared protein fractions from wheat germ and gluten, and then evaluated their immunological activities, in order to find a good protein source for health foods and pharmaceutical applications.

## 2. Materials and methods

### 2.1. Materials

DWG flour was provided by Mantianxue Co., Ltd. (Anyang, China). Wheat gluten flour was a gift from Huahui Biological Industrial Co., Ltd. (Guangzhou, China). RAW 264.7 murine macrophage cells were a gift from the Traditional Chinese Medicine Hospital of Guangdong, and female BALB/c mice were purchased from Zhongshan School of Medicine, SYSU (Guangzhou, China).

### 2.2. Protein fractionation of DWG and wheat gluten

All protein fractions were prepared according to the method of Osborne (1916) with minor modifications; the procedure is shown in Fig. 1A and B. The protein content of each fraction was determined by micro-Kjeldahl method, using protein conversion factors (N) of 5.45 and 5.7, respectively, for wheat germ and wheat gluten.

### 2.3. SDS-PAGE

The SDS-PAGE analysis was performed according to Zhu et al. (2006), with minor modifications. Specifically, 10 μL of protein fraction was mixed with 2 μL of 5 × sample buffer (Bio-Rad, USA), and the mixture was boiled for 5 min before loading onto a gel. Electrophoresis was conducted in a discontinuous system with 12% separating gel and 5% stacking gel. The gel was stained with Coomassie brilliant blue R-250 (Sangon Biotech, China) for 2 h and destained overnight.

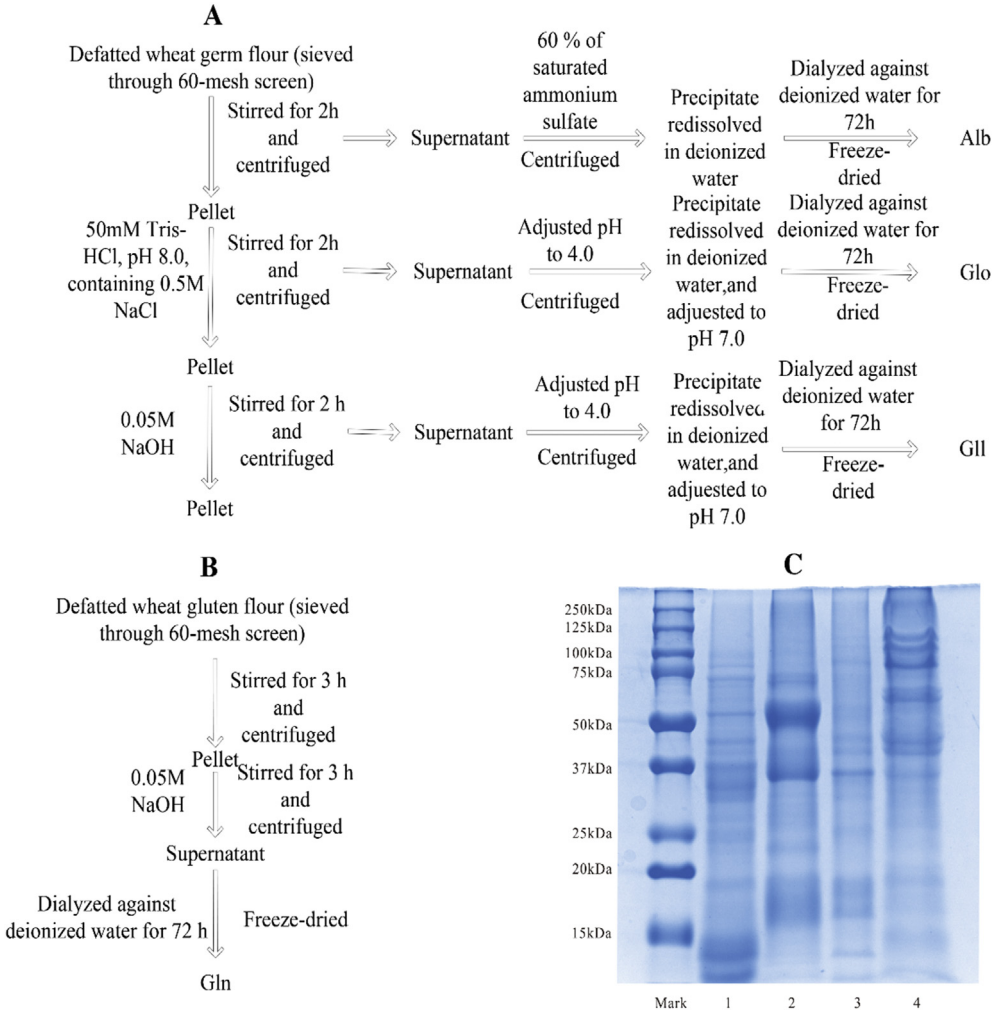


**Fig. 1.** Flow chart depicting the preparation of Alb, Glo and Gll from wheat germ (A) and of Gln from wheat gluten (B). SDS-PAGE profiles of four protein fractions in wheat germ and wheat gluten: lane 1, Alb; lane 2, Glo; lane 3, Gll; lane 4, Gln (C). Alb refer to albumin, Glo refer to globulin, Gll refer to glutelin and Gln refer to glutenin.