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Use of dry-moist heat effects to improve the functionality, immunogenicity of whole wheat flour and its application in bread making

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ABSTRACT

Whole wheat flour samples having protein content of 8.9% and 10.6% were subjected to dry and moist heat conditions to improve the functionality. Dry heat treated flours (DHTF) had higher values of falling number and SDS sedimentation values when compared to moist heat treated flour (MHTF). MHTF showed decrease in water absorption from 75.4 to 56.7%, increase in dough development time from 3.3 to 11.9 min, increase in peak viscosity and cold paste viscosity from 467 to 778 BU and 678 to 1017 BU respectively when compared to untreated flour. MHTF lost its elasticity, SDS-page gel electrophoresis indicated the change especially in the region of gliadin and ELISA indicated 41% reduction in immunogenicity against gliadin. The specific volume of breads prepared from MHTF was significantly lower whereas the crumb firmness value was higher than breads from untreated flours. Breads from treated flours also showed reduction in immunogenicity against gliadin.

1. Introduction

Wheat is a staple food for most of the world's population. The dietary importance of wheat flour is due to its unique visco-elastic property. This arises when the flour is mixed with water to a visco-elastic dough and processed into leavened products and pasta (Gabriella et al., 2001). Wheat plays a major role in feeding the world community but cannot be used without some kind of processing. The conversion of wheat to useful products is an on-going concern of the wheat industries and has thus brought about significant advances in wheat technology (Hansen et al., 1975).

When wheat is consumed as food can affect either adversely or beneficially to the human health and physiology due to the several proteins (Gabriella et al., 2001). Any food protein may be allergenic to mucosa and then evoke an immune (allergic) response. The protein loses its tertiary structure due to high temperature and many of the sites recognized by antibodies on the native molecule are also destroyed (Davis and Williams, 1998).

Some individuals exhibit immune sensitivity to both soluble and insoluble fractions of the gluten proteins. Gluten enteropathy or celiac disease may be caused by an inappropriate immune response to dietary wheat gluten or similar proteins of barley or rye (Olexova et al., 2004). As the prevalence of celiac disease is increasing worldwide, in recent years, there has been increase in consumer interest for wheat free foods (Moore et al., 2004). The components of food that elicit these abnormal immune responses are typically naturally occurring proteins in the foods. Studies on the prevalence of food allergy have shown that the clinical manifestations of food reactions are most commonly observed in the first three years of life (Susanna and Prabhasankar, 2011). Wheat allergy is most frequent in children and infants, and wheat being one among six most commonly implicated allergen. (Scibilla et al., 2006). Celiac disease, baker's asthma, atopic dermatitis and food-dependent exercise induced anaphylaxis are four kinds of symptoms of adverse reactions to wheat flour (Tanabe, 2008).

Several methods have been adopted to bring down the allergenicity to cater to the needs of allergic patients. To induce physical and chemical alterations to the structure of proteins several technological processes have been utilized (Man and Bada, 1987). Thermal processing can be a part of procedure for making hypoallergenic food as heat treatment can bring about substantial changes in their allergenic nature (Davis and Williams, 1998). Thermal processing is not only carried out to reduce allergenicity; but it is to build or enhance texture, flavour, digestibility, microbiologic safety and freedom from toxins (Kilara and Harwalkar,

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1996; Boye et al., 1997; Marin et al., 1992). Conformational changes of heat treated proteins could influence their immunochemical reactions. Rumbo et al., 1996 reported that short conventional heating of gliadins at 90 or 100 °C induced a small increase in their immunoreactivity, while longer exposure or higher temperatures caused an inverse effect.

Hansen et al. (1975) studied the heat-moisture effect on wheat flour and observed that aggregation of lower-molecular weight proteins, disappearance of albumins and globulins, release of peptides and destruction of lysine, arginine and cystine-cysteine. The effect of bromelain on wheat flour to produce hypoallergenic bread was studied by Tanabe et al. (1996) and found that bromelain was effective in decomposing the wheat glutenin IgE-binding epitope structure.

Proteins which have been exposed to irradiation present distinct structural modification due to aggregation, fragmentation and amino acids modification (Davies, 1987; Davies et al., 1987). Irradiation also affected the structural and antigenic properties of proteins as reported by Kume and Matsuda (1995) and Byun et al. (2002). Watanabe et al. (1994) reported that the enzyme treated soft flour retained a certain degree of dough characteristics which could be used as a functional food for patients allergic to wheat. Consuming hypoallergenic cupcakes over a long period showed that more than half of patients became hyposensitive and were later able to eat normal wheat products. This suggests that the hypoallergenic wheat flour can act as anti-allergenic via allergen specific immune tolerance (Tanabe, 2008).

The present study was aimed to produce hypoallergenic flour by adopting simple processing techniques and to evaluate flour and product for immunological, biochemical, rheological, micro structural and baking characteristics.

2. Materials and methods

2.1. Raw materials

Two *Triticum aestivum* wheats namely mixed wheat (Flour A – 8.9% protein) and medium hard wheat (Flour B- 10.6% protein) were procured from the local market. Wheat samples were ground to whole wheat flour in *chakki* mill keeping the grinding conditions similar to get uniform particle size distribution in the flours. The flours were stored at 4 °C until further analysis.

2.2. Processing of whole wheat flour

2.2.1. Dry heat treatment

Whole wheat flour was uniformly spread into a thickness of 1–1.5 cm in aluminium trays and dried in baking oven for 2 h at 100 °C to obtain dry heat treated flour (DHTF), cooled and packed in poly-propylene pouches until further analysis.

2.2.2. Moist heat treatment

Whole wheat flour was spread into a thickness of 2.5–3 cm on wire mesh circular containers of 8 inch diameter lined with butter paper and steamed at atmospheric pressure for 30 min with retention of 10 min time. Flour was then cooled for 30 min and spread uniformly to a thickness of 1.5–2 cm in aluminium trays and dried in an air circulated oven at 60 °C for 2 h. Moist heat treated flour (MHTF) was cooled to room temperature, sieved and stored in poly propylene pouches until further analysis.

2.3. Physico-chemical characterization of flour samples

Dry and Moist heat treated flours of both the wheat varieties along with control samples were analysed for moisture, ash, crude protein, falling number, SDS sedimentation value as per the standard AACC methods. The colour of flour and bread samples was measured in terms of lightness (L) and total colour difference (ΔE) using the Hunter Lab Colour Measuring system (Colour Flex-EZ-Hunter Lab, USA).

2.4. Rheological characterization of flour samples

Flour samples were analysed for farinograph characteristics for evaluation of water absorption and dough stability according to AACC method 54-21. Pasting characteristics of flour samples were studied using Brabender-Visco-Amylograph (Brabender OHG, Duisburg, Germany) according to AACC method.

2.5. Bread making method

Breads were prepared using treated flours along with control flour and evaluated according to the method described by Indrani et al. (2010). Breads were evaluated for specific volume, colour, appearance, crumb grain and overall quality on 9 point hedonic scale. Crumb firmness was measured according to AACC (2000) procedure using texture analyzer (Model Tahdi, Stable Micro-systems, Surrey, U.K.) under the following conditions: sample thickness, 25 mm; load cell, 10 kg; plunger dia, 36 mm and plunger speed, 100 mm per min. Breads were also evaluated for overall quality score, colour and *in-vitro* starch digestibility and *in-vitro* protein digestibility.

2.6. In vitro starch digestibility

In vitro starch digestibility was analysed according to the method of Goni et al. (1997) with some modification. About 50 mg of freeze dried flour and bread samples were incubated with amyloglucosidase (Sigma Chemicals, USA) in acetate buffer (pH 4.6) at 60 °C for 30 min. The sample was inactivated by boiling for 15 min and centrifuged at 1500 rpm for 10 min. The obtained supernatant was made upto 15 ml and 20 μl of it was incubated with 1.5 ml GOD POD (Glucose Oxidase Peroxidase-Span Diagnostics Ltd, India) reagent for 10 min at 37 °C, followed by the addition of 1.5 ml of distilled water. GOD oxidizes glucose to gluconic acid and hydrogen peroxide. Hydrogen peroxide in the presence of peroxidase enzyme couples with Phenol and 4- Aminoantipyrine to form Quinoneimine dye. The colour thus developed was measured at 505 nm. Percentage of glucose release was converted to starch by multiplying glucose percentage with 0.9.

2.7. In vitro protein digestibility

The freeze-dried flour and bread samples were incubated with pepsin and pancreatin according to the method described by Akeson and Stahmann (1964), with slight modifications. Freeze-dried samples of about 2 g were incubated with 1.5 mg of pepsin (Sigma Chemicals, USA) in 15 ml of 0.1 N HCl at 37 °C. After 3 h, the samples were neutralized with 0.5 N NaOH. The sample was incubated with 4 mg pancreatin (Sigma Chemicals, USA) in 0.2 N phosphate buffer (pH 8) and 0.005 N sodium azide was added. Samples were incubated overnight; 1 ml of 10% TCA was added and centrifuged at 3000 rpm for 20 min. The nitrogen content in supernatant was estimated by Kjeldahl method.

2.8. Microstructural characterization of flour and bread samples

Defatted and freeze-dried flour and bread samples were scanned under scanning electron microscope (LEO 435 VP, USA) according to the method described by Prabhasankar et al. (2009). The