Effect of Thermal Processing of Dietary Fibers on the Bioavailability of Proteins from Wheat and Barley Flour

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Abstract
Changes in particle size, solubility and chemical structure of different fiber components can cause changes in the bioavailability of some nutrients. Numerous studies have shown a reduction in apparent digestion of proteins in the gastrointestinal tract caused by direct and indirect processes. Endogenous nitrogen losses are due to increased secretion of digestive juices and increased desquamation in the presence of dietary fiber. Food fibers reduce digestion of food and endogenous proteins. Food processing can improve the bioavailability of proteins. Reduction of thermally latent antinutritional factors, such as trypsin inhibitors, phytates and polyphenols, as well as partial denaturation of proteins by heat treatment contributes to their digestibility. Protein quality in processed foods decreases primarily due to reduced lysine bioavailability. Experiments revealed an increase in protein digestibility (PD) in whole wheat flour heated samples in a forced air oven at 150°C over 0-10 minutes: 31.8% at time 0, 43.73% at 5 minutes and 48.43% at 10 minutes of heating. When heating for 15 minutes, digestibility decreased to near the unprocessed sample value (33.8%). PD in wheat samples exposed to microwave for 30, 60 and 90 seconds increased throughout the entire time period, with a maximum of 45.06% when exposed for 90 seconds. PD increased moderately in whole barley flour samples heated at 150°C, from 27.46% for the unprocessed sample to a maximum of 33.47% for the 5 minutes heated sample. PD in barley samples exposed to microwave was higher than in the unprocessed sample, with a maximum of 36.34% for 30 seconds exposure.

Keywords: wheat, barley, thermal processing, protein digestibility

1. Introduction

When predicting the nutritional quality of feeds, information on the digestibility of the various nutrients is of utmost importance. Digestibility is a measure of the availability of nutrients. The biological availability of nutrients is of great importance in formulating a balanced ration to attain maximum productivity in animals.
Direct determination of energy values of feeds by *in vivo* trials is expensive and time-consuming; it also requires animal facilities and relatively large amounts of experimental diets. Therefore *in vitro* procedures were developed.

Only the soluble portion can be taken up into the circulation and used in supplying the animal body with material for building and repair of tissue or supply the energy necessary for body functions. Digestibility measurements are easier to obtain than intake measurements, and therefore nutritionists made considerable effort to develop effective means of determining digestibility [1]. Measurement of *in vitro* protein digestibility (PD) has a high degree of correlation to *in vivo* digestibility [2,3]. The *in vitro* techniques [2,3] simulate the digestion process, using either an inoculum prepared from pig digestive contents [4] or enzymatic preparations [5]. The *in vitro* methods are based on consecutive incubations with pepsin and pancreatin, containing all the

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necessary enzymes for solubilizing the potentially digestible nutrients [6-11].

The digestibility of proteins depends on protein structure (amino acid profile, protein folding and crosslinking) and on external factors (pH, temperature and ionic strength conditions, the presence of secondary molecules such as emulsifiers and antinutritional factors). Factors affecting protein solubility also influence protein digestibility. Dietary fiber (DF) has also been associated with hampered protein hydrolysis. However, the effect of DF may be a purely physical one, i.e., by increasing the viscosity of the gastrointestinal tract content, hydrolytic enzymes may not as quickly diffuse to and gain access to their substrates [12,13]. Previous researches show that DF increased *in vivo* intestinal viscosity and slowed down the rate of diffusion of substrates, digestive enzymes, and final products of digestion, and therefore affect the digestion and utilization of nutrients [14,15].

Food processing has a substantial effect on these factors and, hence, on protein digestibility.

Cereal proteins are in a tightly folded conformation that is hampering their digestibility. In addition, proteins often occur in supramolecular structures such as protein bodies [16,17] and/or are physically entrapped in cellular structures [18] that restricts the access of the hydrolytic enzymes to their substrates. The processing of food serves several purposes, such as ensuring food safety, prolonging shelf life and increasing digestibility. Food processing triggers alterations in molecular and supramolecular structures to allow digestive enzymes to gain easier access to the nutrients/biopolymers, hence, improving food digestibility. Thermal processing usually leads to conformational changes and, if severe enough, even to irreversible loss of protein folding or molecular scission. Denaturation modifies the protein structure, making it more accessible for hydrolytic enzymes, and, hence, increases protein digestibility. Protein denaturation also exposes hydrophobic sites of the proteins that are otherwise shielded from the aqueous environment. The hydrophobic effect will also lead to protein aggregation and decreased digestibility [19]

Food processing is meant to increase the nutritional value of food products, by making the biopolymers more readily available for digestive processes [20]. In cereal food products the gelatinization of starch, disruption of cell walls and the inactivation of toxic and antinutritional factors, need to be achieved to ensure proper energy and nutrient extraction.

Changes in particle size, solubility and chemical structure of different fiber components can cause changes in the bioavailability of some nutrients. Numerous studies have shown a reduction in apparent digestion of proteins in the gastrointestinal tract caused by direct and indirect processes. Endogenous nitrogen losses are due to increased secretion of digestive juices and increased desquamation in the presence of DF. Cereal grains contain various amounts of DF depending on the species and tissue type [21]. Food fibers reduce digestion of food and endogenous proteins.

Food processing can improve protein bioavailability. Reduction of thermally latent antinutritional factors, such as trypsin inhibitors, phytates and polyphenols, as well as partial denaturation of proteins by heat treatment contributes to their digestibility. Protein quality in processed foods decreases primarily due to reduced lysine bioavailability. DF, an ubiquitous component of plant foods which includes materials of diverse chemical and morphological structure, reduces protein digestibility. Thermal treatment can modify both the chemical composition and the physical properties of DF and thus can improve PD [22].

The study was conducted to evaluate the effect of thermal processing on *in vitro* PD of wheat and barley flour.

2. Materials and methods

Wheat and barley grains were milled to 0.5 mm granulation and heated in a forced air oven or by microwave radiations for different time periods. The *in vitro* digestion consisted of a two step enzymatic procedure: (1) initial simulation of gastric digestion by pepsin and (2) simulation of small intestine digestion by pancreatin according to the method of Boisen et al. [5] with some modifications [23].

For the gastric and intestinal digestion, 4 g material was weighed with an accuracy of 0.1 mg into a 50 mL plastic centrifuge tube. After 120 minutes gastric digestion of the sample, 2 ml phosphate buffer (0.2 M, pH 6.8), 2 ml of 0.6 N NaOH (to adjust pH to 6.8), and 2 ml 2%

pancreatin (Sigma P7545) were added to the mixture.
The tubes were incubated in the water bath with shaking at 120 rpm at 37°C for 240 minutes. Samples were then centrifuged at 5000g for 10 minutes. Residue was dried for 16 h at 100°C and analyzed for DM solubility and CP content.
All samples for *in vitro* analysis were done in duplicate.
Crude protein (CP) was determined by the macro-Kjeldahl technique (%N× 6.25) [24].
The *in vitro* PD was calculated from the difference between concentrations in the sample and the undigested residue.
PD (g%) = (CP cereal - CP undigested)/(CP cereal) x100.

Statistical analysis
Average values, standard deviations, and variance coefficients were calculated. For continuous variables, the regression analysis was performed and the determination coefficients R^2 have been calculated. The results were statistically analyzed using the t-test. Significant differences were reported when $P \leq 0.05$.

3. Results and discussion

Experiments revealed an increase in protein digestibility (PD) in whole wheat flour heated samples in a forced air oven at 150°C over 0-10 minutes: 31.8% at time 0, 43.73% at 5 minutes and 48.43% at 10 minutes of heating. When heating for 15 minutes, digestibility decreased to near the unprocessed sample value (33.8%). PD increased moderately in whole barley flour samples heated at 150°C, from 27.46% for the unprocessed sample to a maximum of 33.47% for the 5 minutes heated sample (Figures 1 and 3).

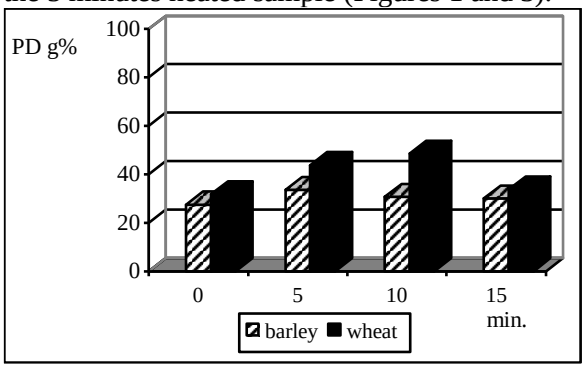

Figure 1. *In vitro* PD of thermal processed wheat and barley

PD in barley samples exposed to microwave was higher than in the unprocessed sample, with a maximum of 36.34% for 30 seconds exposure.
PD in wheat samples exposed to microwave for 30, 60 and 90 seconds increased throughout the entire time period, with a maximum of 45.06% when exposed for 90 seconds (Figures 2 and 4).

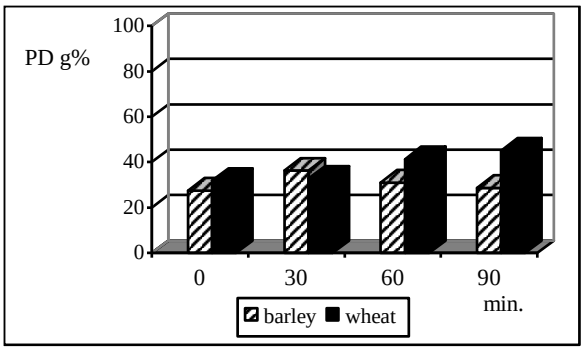

Figure 2. *In vitro* PD of microwave processed wheat and barley

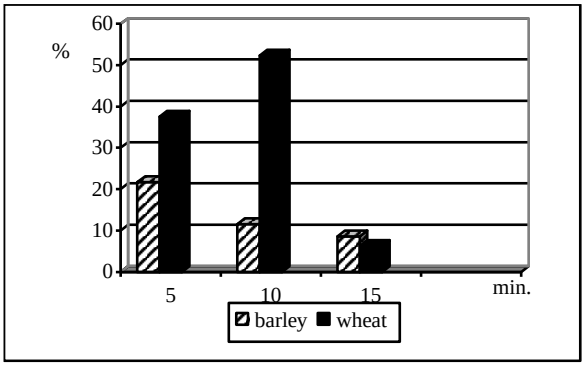

Figure 3. Increase of *in vitro* PD after thermal processing of wheat and barley

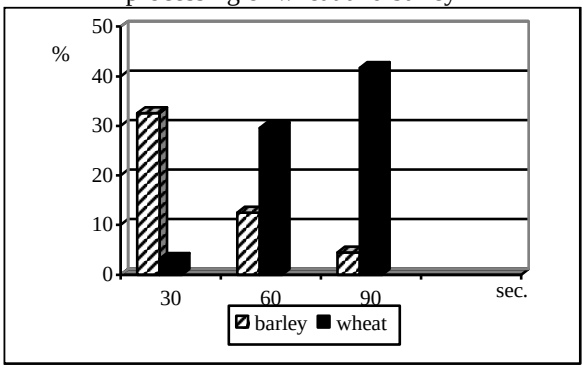

Figure 4. Increase of *in vitro* PD after microwave processing of wheat and barley

4. Conclusions

Short thermal processing modifies the physical and chemical structure of DF, facilitating accessibility of enzymes to proteins and

improving their bioavailability. The solubility of the fibers and especially the gelatinization of the starch can improve the digestibility of the proteins.

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