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Dry Roasting of Beans

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Dr. Irving Liener, University of Minnesota, Minneapolis, MN, and Grant Kuhn of the Grant L. Kuhn and Co., Saginaw, MI, reviewed a new process for dry-bed roasting of navy beans. The work is reported in detail in an article by Yadav and Liener, Legume Research 1 (1):17 (1977). The procedure appears applicable to many seeds including soybeans that need heat treatment prior to consumption by animals or humans. The seeds are roasted for a short time at ca. 200 C before being processed to flour or retained as seeds. By means of a screw conveyor, beans are dropped into an inclined rotating drum. It contains sand, salt or ceramic pellets as a heat transfer medium held at 196-204

C. The beans are retained for ca. 20-25 seconds. Beans are dropped into a separator which permits transfer of the salt, sand, or pellets back to the rotating drum heater. Roasted beans may be ground to flour, converted to other products or marketed as "roasted" beans. Tests showed the beans to have digestibility and PER superior to autoclaved beans. Trypsin inhibitor was reduced ca. 75% to 4×10^{-3} units per g and hemagglutinin units to 0.2. With added methionine, the PER was 3.1 ± 0.08 and with 50-50 corn-beans, the PER was equal or better than casein. Tests are currently being completed on soybeans.

Physiological Effects of High Soybean Diet in Man

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Two groups of 46 human volunteers were fed a control or a soy diet for 4 weeks and the diet switched for another 4 weeks. The soy diet was based on a soy concentrate of ca. 62% protein. The nutritional, medical and toxicological experts of the group involved in this study concluded unanimously that changes in physiological parameters were not abnormal. Where significant differences were noted, they fell within normal clinical limits. No undesirable effect was noted with no indication of any undesirable long term effect. Properly heat-treated soy protein products should not give any health problems with humans. See Cereal Foods World 23(5):234 (1978).

Toxicity of Cereal Protein - Derived Peptides for in Vitro Developing Intestine from Rat Fetus

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ABSTRACT

This paper describes work that shows that the PTC digest of gliadins extracted from hexaploid wheat flour have the same toxic activity of the PTC digest of gliadins extracted from hexaploid wheat gluten. Moreover, not all wheat species apparently contain the toxic components, thus suggesting that durum wheat foods may present, as compared to soft wheat foods, a lower risk for human health under particular circumstances. Similar considerations seem to apply to other cereal genera that seem to differ with respect to the presence and/or content of toxic peptides. Further experiments to test such a working hypothesis are now being made.

INTRODUCTION

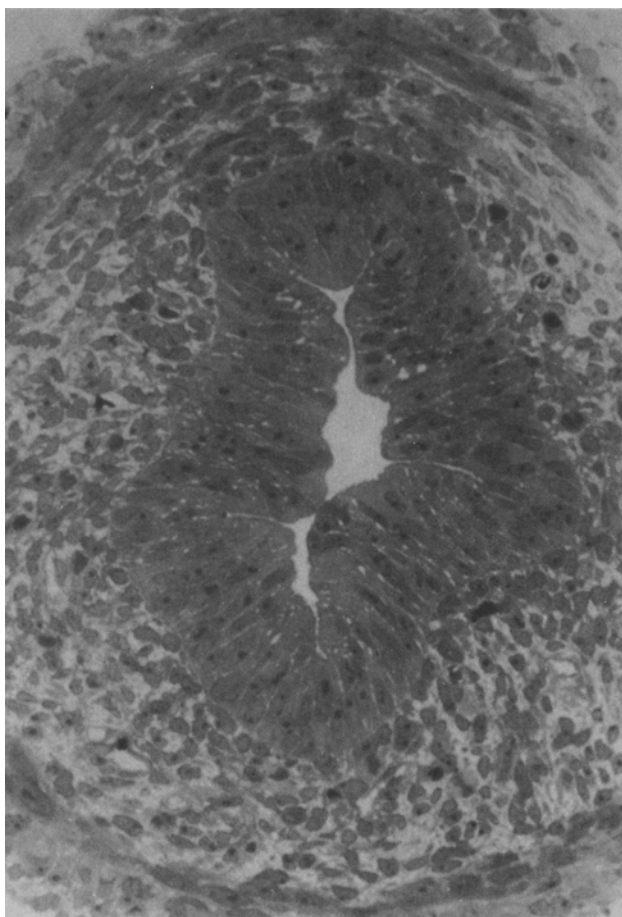Many factors, such as protein content, essential amino

FIG. 1. Jejunal mucosa from a 17-day rat fetus before culture. No villi are present and the epithelium consists of stratified undifferentiated cells. Richardson's stain (x310).
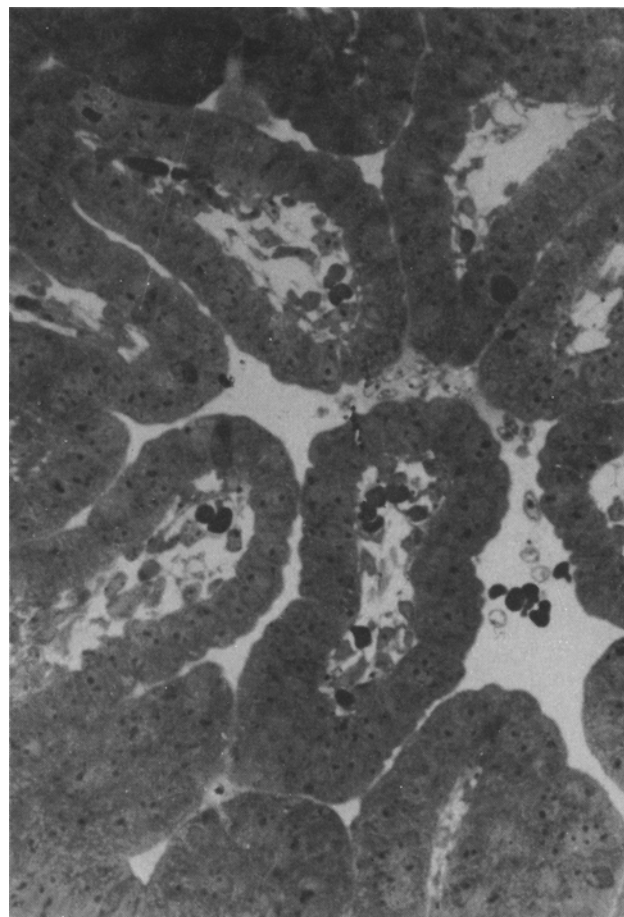

FIG. 2. Jejunal mucosa from the same fetus as Fig. 1 which had been cultured for 48 hr showing well developed villi lined by a single layer of relatively differentiated columnar epithelial cells. Richardson's stain (x310).

acid composition and availability, protein digestibility and other factors contribute to the poor nutritional value of cereal proteins as compared to proteins from animal sources (1). Moreover, some cereal species contain chemicals of different nature which can have serious effects under particular circumstances (i.e., tannins in a low protein diet, phytates in a metal-deficient diet) (1). These factors affecting nutritional value of cereal and other plant proteins are now well established, and nutritionists as well as food technologists have learned how to deal with such problems.

However, there are other toxic components in some cereal species (and they might as well be present in other plant materials) that act under special circumstances and that we do not know yet how to inactivate or counterbalance. A typical relevant condition in this respect is coeliac disease, a gluten-induced entheropathy that affects sensitive humans (perhaps 1 over each 1,000 people) (2). It has been reported (3) that patients with coeliac disease show intolerance not only of wheat, but also of rye, barley and oats. The role of wheat gluten as a toxic factor in the pathogenesis of coeliac disease is now well established, and it has been suggested that gliadin, the ethanol soluble fraction of gluten, is in fact responsible for the adverse effects observed when gluten is present in the diet of coeliac individuals (4,5). Not only whole gliadin proteins, but also peptides deriving from proteolytic digestion of wheat gliadins are toxic in coeliac disease (6-9).

Moreover, there are indications that wheat gluten or gluten fractions might have a harmful role in other types of small intestinal diseases. Bayless and Swanson (10) reported that patients with tropical sprue responded to a gluten-free diet with a decreased steatorrhea and with an improvement in the jejunal lesions. Hedberg et al. (11) described patients

with postgastrectomy steatorrhea that was improved by a gluten-free diet. Levine (12) found that subjects convalescing from Laennec's cirrhosis, tuberculosis, or viral hepatitis showed a significant increase in fecal fat excretion upon addition of 100-150 g gluten daily to the diet, whereas no effect was observed with normal individuals. Rudman et al. (13) showed that patients with regional enteritis given 5 to 50 g of gluten with the diet for 12 days suffered gastrointestinal bleeding, increased steatorrhea, fever, diarrhea and abdominal discomfort due to inclusion of gluten in the diet. Temporary gluten intolerance (14) as well as gluten sensitive diarrhea without evidence of coeliac disease (15) have also been described.

The detection and characterization of cereal components which may be toxic for man under some conditions is very difficult because of the lack of suitable in vivo or in vitro methods for toxicity testing. This applies to compounds responsible for coeliac disease as well as to those involved in other intolerances to cereal proteins. Moreover, the possibility exists that some new protein sources also contain similar compounds which may exert a toxic effect on man. At the moment we have no way of testing such a possibility to give good indications to food technologists for removal and/or inactivation of toxic compounds. Therefore, we are trying to develop an in vitro system to be used for identification of this special type of toxic compounds. A common feature of cereal intolerance pathologies is the increase of enterocyte turnover resulting in the presence of immature enterocytes on the surface of the mucosa. As such a condition could be at least in part assimilated to some biological stages underlying maturation of rat fetal intestine, which takes place in vitro in a way
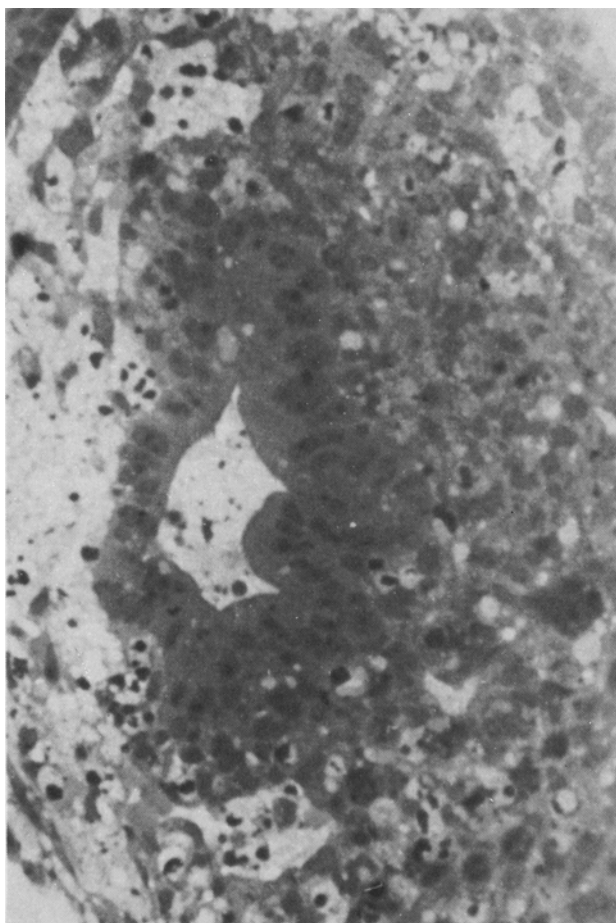
FIG. 3. Jejunal mucosa from the same fetus as Fig. 1 which had been cultured for 48 hr in the presence of the PTC digest of gliadin prepared from hexaploid wheat (0.1 mg/ml). No villi are developed, and the intestinal surface is lined by a single layer of relatively undifferentiated cuboidal and columnar epithelial cells. Large lysosome-like inclusions are present. Richardson's stain (x310).
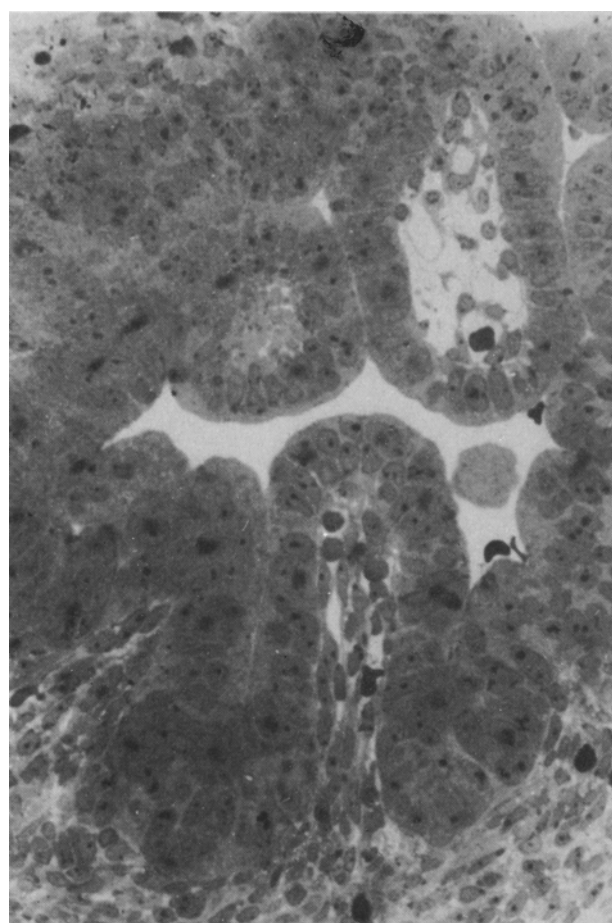

FIG. 4. Jejunal mucosa from the same fetus as Fig. 1 cultured for 48 hr in the presence of the PTC digest of gliadin prepared from tetraploid wheat (0.5 mg/ml). The maturation of the jejunal mucosa happens normally. Well formed villi similar to those shown in Fig. 3 are present and their epithelial lining consists of a single layer of fairly differentiated columnar cells. Richardson's stain (x 310).

comparable to what happens in vivo (16), we have undertaken a research program to evaluate whether such a tissue provides a good model for the study of toxic proteins and peptides. In a previous study (17) we prepared a peptic-tryptic-cotazym (PTC) digest of a crude gliadin preparation from hexaploid wheat gluten under experimental conditions simulating in vivo protein digestion by humans; this digest was then fractionated into 10 peaks by ion exchange chromatography. PTC gliadin digest and one of its subfractions (coded as fraction 9 according to its elution pattern) were very active in inhibiting in vitro development and morphogenesis of small intestine from 17 and 18 day old rat fetuses, but had no toxic effect in the culture of jejunum from 21 day old rat fetus. In this previous study (17), we also tested several wheat albumin and gliadin fractions on in vitro developing small intestine from 17 day old rat fetus. Among all the tested protein fractions, only one gliadin fraction (coded as α_{10} gliadin from its gel electrophoretic mobility) exhibited a toxic effect; morphological alterations induced by α_{10} gliadin were similar to those induced by PTC digest and fraction 9.

We have carried out further experiments to investigate the effects on such a tissue system of peptides obtained through digestion of several protein fractions from hexaploid and tetraploid wheats as well as from other cereal species.

EXPERIMENTAL

For preparation of the globulin fraction, 100 g of finely ground whole cereal flour was extracted at 4 C for 3 hr in a shaker with 11 of a 0.04 M Na_2HPO_4 (pH 7) buffer containing 1.8 M $(NH_4)_2SO_4$. After extraction, the suspension was centrifuged for 15 min at 16,000 x g. The supernatant was dialyzed against distilled water for 48 hr at 4 C to obtain the globulin fraction as a precipitate. Identical experimental conditions were applied with other solvents for the consecutive extraction of the albumin, gliadin, and glutelin fractions from the residue from the first extraction. The solvent for extraction of albumins was 0.04 M Na_2HPO_4 (pH 7) containing 0.4 M $(NH_4)_2SO_4$, that for extraction of gliadins was ethanol/water (70:30 v/v), and for the extraction of glutelin 0.1 M acetic acid was used. Gliadin and glutelin fractions were extracted at room temperature using 300 ml of solvent for 100 g of flour.

The procedure for gliadin preparation used here is significantly different from that previously used by de Ritis et al. (17) mainly because they extracted gliadins from commercial wheat gluten instead of wheat flour.

Peptic-tryptic-cotazym digests were prepared from cereal protein fractions following the three steps procedure of Bronstein et al. (18) as reported by de Ritis et al. (17). One hundred g of protein fraction was digested in 11 of 0.2 N NCl (pH 1.8) with 2 g of purified pepsin at 37 C for 2 hr. The resultant peptic digest was further digested by addition of 2 g of purified trypsin after pH adjustment to 8.0 with 2 N NaOH. The reaction mixture was vigorously stirred at 37 C for 4 hr at pH 8.0. Then, the peptic-tryptic digest was treated with 2 g of purified cotazym and mechanically stirred for 2 hr at pH 8.0. During all the digestion procedure, the pH was checked periodically and, when

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TABLE I

Morphological Features of Maturation of Rat Fetal Jejunum Cultures
in Vitro in the Absence of in the Presence of Gliadin Peptides

Sample	Villi			Epithelium				Goblet cells	Degenerative changes[a]	
				Stratified	Monostratified					
	Absent	Rudimentary	Present		Cuboidal	Columnar	Mixed	Present	±	++
Uncultured 17-day fetuses (42)[b]	42			42				---		
After 48 hr culture without gliadin peptides (42)[b]	2	1	39	---		41	1	27	10	2
After 48 hr culture with PTC-gliadin digest from hexaploid wheat (0.5 mg/ml) (4)[b]	4				2	1	1	---	3	1
(0.1 mg/ml) (6)[b]	6				2	2	2	1	3	---
After 48 hr culture with PTC-gliadin digest from tetraploid wheat (0.5 mg/ml) (6)[b]		1	5			6		2	2	1
(0.1 mg/ml) (6)[b]			6			6		3	2	---

[a]± Indicates slight degenerative changes; ++ indicates large necrotic areas.

[b]Number of fetal jejunal cultured.

needed, adjusted with HCl or NaOH. At the end of the whole digestion procedure, the digest was submitted to gel filtration and the peptide fractions eluted after cytochrome c were collected and freeze-dried. This enzyme-free, low molecular weight peptide pool has been coded as PTC protein digest. Moreover, a PTC bovine serum albumin digest to be used as a control was prepared under identical experimental conditions.

For in vitro culture of fetal jejunum, time-pregnant Wistar rats were anesthetized with ether and 17 day fetuses were removed at laparatomy. Fetal jejunum segments were isolated and cultured in vitro for 48 hr in a serum-free medium, according to the method described by de Ritis et al. (17). Jejunal segments from the same fetus were cultured in the absence and in the presence of tested peptides. Differentiation of the fetal rat jejunum was followed morphologically by light microsopy examination as reported by de Ritis et al. (17).

RESULTS AND DISCUSSION

As indicated by light microscopy examination, jejunal segments from seventeen day old rat fetuses did not show before in vitro culture any villus and only undifferentiated cuboidal stratified epithelium lining the jejunum was present (Figure 1). Moreover, no goblet cells could be detected at this stage of tissue development. After 48 hr in vitro culture (Figure 2), there were clear morphological evidences of tissue maturation. Well differentiated villi were present in cultured jejunal segments. The epithelial lining consisted exclusively of simple columnar epithelium; goblet cells were also identified (Table 1). The peptic-tryptic-cotazym digest (PTC digest) of the gliadin fraction prepared from hexaploid (Triticum aestivum) wheat was very active in slowing down in vitro development of fetal rat intestine and in increasing occurrence and severity, especially at the mesenchyme level, of degenerative changes sporadically observed in the control tissue (Figure 3). Such effects were also observed at the lowest peptide concentration tested

(0.1 mg/ml of incubation medium) (Table 1).

No one of the effects induced by the PTC gliadin digest from hexaploid wheat was observed with the PTC digest of gliadin fraction obtained from tetraploid (Triticum durum) wheat, even when it was tested at a concentration as high as 0.5 mg/ml (Figure 4 and Table 1).

Preliminary experiments have also been carried out with PTC gliadin digest from diploid (Triticum monococcum) wheat as well as from other cereal species including rice, barley, oats and rye. Preliminary data available seem to indicate that the digests from rye and oats, although to a different extent, have the toxic activity, whereas the digests from diploid wheats seem inactive. A few experiments have also been carried out to test PTC digests of hexaploid wheat albumin, globulin and glutelin fractions; in all the cases no toxic activity has been detected up to now.

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