

# Modifications to Physicochemical and Nutritional Properties of Hard-To-Cook Beans (*Phaseolus vulgaris* L.) by Extrusion Cooking

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The objective of this work was to evaluate extrusion cooking as a means to improve the nutritional properties of *Phaseolus vulgaris* L. that had been stored either at 42 °C and 80% relative humidity for 6 weeks or for periods >1 year in cereal stores in tropical conditions. Storage under these conditions resulted in an increase in cooking time increased (7.7- and 12-fold, respectively) as a result of development of the hard-to-cook (HTC) defect. Single-screw extrusion of the milled beans was carried out at four barrel temperatures and two moisture contents. The extrudate bulk density and water solubility index decreased with increasing temperature, whereas the water absorption index increased due to the higher proportion of gelatinized starch in the extruded samples. Both fresh and HTC beans contained nutritionally significant amounts of lectins, trypsin, and  $\alpha$ -amylase inhibitors, which were mostly inactivated by extrusion. Extrusion also caused a considerable redistribution of insoluble dietary fiber to soluble, although the total dietary fiber content was not affected. Changes in solubility involved pectic polysaccharides, arabinose and uronic acids being the main sugars involved. Stored beans subjected to extrusion cooking showed physical and chemical characteristics similar to those of extrudates from fresh beans.

**Keywords:** *Extrusion cooking; hard-to-cook beans; physical properties; antinutrients; dietary fiber*

## INTRODUCTION

Legumes are one of the most important sources of food in developing countries, especially in terms of protein (Molina et al., 1977). Of the legumes, dry beans are a major source of dietary protein, dietary fiber (DF), starch, vitamins, and certain minerals. However, unlike cereals, dry beans may deteriorate very quickly as a function of time and conditions of storage, particularly under conditions of high temperature and high humidity, which are prevalent in tropical countries. The main observed deteriorations involve an increase in cooking time, deterioration of texture and flavor, and loss of nutritive value (Jones and Boulter, 1983). This phenomenon is known as the hard-to-cook (HTC) defect (Molina et al., 1976; Reyes-Moreno et al., 1994). In addition, the nutritive value of beans is limited by the presence of antinutrient factors such as lectins, trypsin inhibitors, and  $\alpha$ -amylase inhibitor.

Relatively few investigations have been carried out relating possible changes in antinutrient compounds in beans to the development of the HTC phenomenon (Martín-Cabrejas et al., 1995a, 1997; Karanja et al., 1996). These antinutrient factors are known to have

deleterious or toxic effects for animals and man (Pusztai and Palmer, 1977; Grant et al., 1983). Therefore, for effective utilization of these beans for human nutrition, removal or elimination of these undesirable attributes by a suitable pretreatment is necessary before they can be safely used as a food source (Grant et al., 1986; Liener, 1989).

In recent years, extrusion cooking has been used increasingly in the production of foods and food ingredients such as breakfast cereals, baby foods, flat breads, snacks, meat and cheese analogues, and modified starches etc (Mercier et al., 1989; Kokini et al., 1992). Extrusion cooking is unique among heat processes in that the material is subjected to intense mechanical shear; moistened starchy or proteinaceous foods are worked into a viscous, plastic-like dough and cooked before being forced through a die. The intense structural disruption and mixing facilitates reactions otherwise limited by diffusion of reactants and products (Asp and Bjorck, 1989). Some results of cooking during the extrusion process are the gelatinization of starch, denaturation of protein, inactivation of many native enzymes, which causes food deterioration during storage, inactivation of antinutrient factors (Rackis et al., 1975; Edwards et al., 1994; Steel et al., 1995), and reduction of microbial counts in the final product (Harper, 1981).

Many extruded products are good sources of DF, and extrusion cooking is a suitable process for the production of fiber-enriched products. In addition, extrusion cooking has been considered as an alternative way of modifying the functionality of dietary fiber (Camire et al., 1990,

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1997; Qian and Ding, 1996). There are a number of methods by which DF can be quantified. The AOAC enzymatic-gravimetric method can be used for obtaining the DF content of plant foods; however, it fails to distinguish between the categories of polysaccharides. Hence, to study the chemical changes that are due to extrusion, the DF measurement must involve carbohydrate analysis of the soluble and insoluble components (Martin-Cabrejas et al., 1995b).

The objective of the present work was to investigate the use of extrusion technology to inactivate deleterious antinutrients and to modify dietary fiber components in beans, with a view to enhancing the utilization of HTC forms in traditional foods and thereby increasing protein levels.

## MATERIALS AND METHODS

**Bean Samples.** Beans (*Phaseolus vulgaris* L.) were obtained from different sources: fresh British-harvested Horsehead (HH) beans from Peas and Beans (Cambridge, U.K.); fresh Canadian Wonder (CW) beans from Thika Experimental Station (Nairobi, Kenya); and very HTC Canadian Wonder beans from the National Cereal Board Stores (Kenya), where they had been stored in sisal bags for periods >1 year and therefore had already developed the HTC defect. Beans after acquisition were stored dry in a cold room (2 °C) until required for use. In fresh HH beans, the HTC defect was induced by storing in a controlled room at 42 °C and 80% relative humidity (RH) for 6 weeks.

**Cooking Time of Whole Beans.** Prior to cooking, 50 beans were soaked for 16 h in deionized water. A Mattson-type cooker (Downie et al., 1997) was used to determine the cooking time of the individual beans, defined as the time taken for the bean to fail to support a weighted rod. Cooking time was reported as the time taken to cook 50% of the beans (CT<sub>50</sub>), because of the distribution of cooking times of individual beans.

**Extrusion Cooking.** Beans were milled on an Alpine 160 Z pin mill (Cambridge, U.K.) to 0.5 mm particle size. Bean flours were extruded using a Brabender 20DN benchtop extruder (South Hackensack, NJ) fitted with a grooved barrel and a 1:1 compression ratio screw. It was flooded fed by hand and the throughput measured for each speed setting. The throughput generally increased from 30 to 90 g min<sup>-1</sup> when the screw speed increased from 49 to 133 rpm. The specific mechanical energy (SME) was measured from the mechanical power consumption and the mass throughput:

$$\text{SME (kWh kg}^{-1}\text{)} = \frac{\text{screw speed} \times \text{power (kW)} \times \text{torque (\%)}}{\text{max screw speed} \times \text{throughput (kg h}^{-1}\text{)} \times 100}$$

The extruder barrel was heated at 140, 160, 170, and 180 °C, and a circular die of 3 mm diameter was used. Bean flours were mixed with water to give 25 and 30% moisture contents (MC) and equilibrated over a period of 4 h prior to extrusion. Extrudates were dried to equilibrium at room temperature for 5 days and then milled using the Alpine 160 Z pin mill and sealed in plastic bags, cooled to room temperature, and stored for 24 h.

**Physical Properties.** The bulk density of related extrudates was calculated from the mass and volume of cylindrical pieces (five replicates). The water absorption and solubility indices (WAI and WSI) were measured using a technique developed for cereals (Anderson et al., 1969) in which the ground product was suspended in water, stirred, and then centrifuged. The supernatant was evaporated to give a dried solids weight, which is the WSI. The WAI is the weight of gel obtained after removal of the supernatant per unit weight of original dry solids.

**Microscopy of Extrudates.** Transverse slices, ~2 mm thick, were cut from extrudates using a razor blade and fixed

in 3% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.4, for 3 h. The slices were dehydrated in an ethanol series with three changes in 100% ethanol and then infiltrated with the acrylic resin LR White (London Resin Co. Ltd., Reading, U.K.). The samples were transferred to gelatin capsules containing fresh resin, which was polymerized for 24 h at 60 °C. Sections, 1–2 μm thick, were cut with glass knives, dried down onto glass slides, and stained with 1% toluidine blue in 1% Borax, pH 11.

**Determination of Antinutrient Factors.** Bean flours or extrudates were extracted (1:10, w/v) by stirring with 0.02 M sodium phosphate buffer, pH 7.0, containing NaCl (8 g/L) overnight at 1 °C followed by centrifugation at 50000g for 25 min. The resultant clear supernatants were used for antinutrient evaluations. Trypsin inhibitor activity was determined according to the method of Grant et al. (1986), based on the method of Kakade et al. (1969), using *N*α-benzoyl-DL-*p*-nitroanilide as substrate. The α-amylase inhibitor content was evaluated according to the starch/iodine procedure of Piergiorganni (1992). For inhibitor levels, triplicate assays were conducted. Hemagglutinating activity (PHA) was estimated in sodium phosphate extracts by a serial dilution procedure using rat blood cells (Grant et al., 1983, 1986). The assays were reproducible to ±1 dilution, and values in the text were the means of four separate measurements.

**DF Determination.** Total (TDF), insoluble (IDF), and soluble dietary fiber (SDF) were determined using the enzymatic-gravimetric AOAC Method 991.43 (1992). The method was based on the enzymatic removal of starch and protein from the material and separation into soluble and insoluble fractions by filtration.

**Chemical Analysis.** IDF and SDF fractions were dispersed in H<sub>2</sub>SO<sub>4</sub> as described in Martin-Cabrejas et al. (1995b). The neutral sugar composition of the DF was determined by HPLC (Marlett and Chesters, 1985) and uronic acid content by an automatic colorimetric method (Esteban et al., 1993). Klason lignin was obtained from the weight of IDF residue left after hydrolysis with 72% H<sub>2</sub>SO<sub>4</sub> for 3 h at 20 °C, followed by dilution to 1 M acid and heating at 100 °C for 2.5 h. The insoluble residue was recovered quantitatively over a glass filter (Pyrex No. 2), washed thoroughly with water, and then dried for 18 h at 105 °C.

**Statistical Analysis.** All analyses were performed at least in triplicate. Results were analyzed by the Statistical Analysis System program (SAS, 1985). When effects of extrusion conditions were shown, the results for each parameter of each variety were evaluated further using Duncan's test. Differences were considered significant at *p* ≤ 0.05.

## RESULTS AND DISCUSSION

**Unprocessed Beans.** The mean cooking times of fresh HH and CW beans were 26 and 25 min, respectively. In contrast, the mean cooking times of their HTC counterparts were 199 and >300 min, respectively, demonstrating clearly the HTC phenomenon (Hentges et al., 1991). Both fresh and HTC beans contained nutritionally significant amounts of lectins, trypsin inhibitors, and α-amylase inhibitor (Table 1). HTC samples had higher contents of lectin than the corresponding fresh beans [4.9 and 9.7 g of PHA equiv/kg of dry matter (dm) in fresh and HTC HH and 9.7 and 19.4 g of PHA equiv/kg of dm in fresh and HTC CW beans, respectively], which is consistent with the results reported by Martin-Cabrejas et al. (1995a). In addition, but to a lesser extent, the trypsin inhibitory activity also increased during the development of the HTC defect. In contrast, α-amylase inhibitor levels in HTC samples were significantly lower than those in comparable fresh beans (3.3 and 2.6 g of inhibitor/kg of dm in fresh and HTC HH and 2.6 and 1.8 g of inhibitor/kg of dm in fresh and HTC CW beans, respectively).

**Table 1. Effect of Extrusion Cooking on Antinutrient Factors in Bean Flours<sup>a</sup>**

bean cultivar	extrusion conditions	trypsin inhibitor (g kg <sup>-1</sup> )	α-amylase inhibitor (g kg <sup>-1</sup> )	lectin (g of PHA equiv kg <sup>-1</sup> )
fresh Horsehead raw		5.9 ± 0.3 <sup>a</sup>	3.3 ± 0.2	4.9 ± 2.4
	25% MC			
	140 °C	0.9 ± 0.1 <sup>b</sup>	nd	nd
	160 °C	0.5 ± 0.1 <sup>c</sup>	nd	nd
	170 °C	nd	nd	nd
	180 °C	nd	nd	nd
	30% MC			
	140 °C	2.4 ± 0.2 <sup>b</sup>	nd	0.2 ± 0.0
	160 °C	0.6 ± 0.1 <sup>c</sup>	nd	nd
	170 °C	0.3 ± 0.0 <sup>d</sup>	nd	nd
HTC Horsehead raw		9.2 ± 0.4 <sup>a</sup>	2.6 ± 0.2	9.7 ± 4.8
	25% MC			
	170 °C	0.9 ± 0.1 <sup>b</sup>	nd	nd
	180 °C	nd	nd	nd
fresh Canadian Wonder raw		5.6 ± 0.4	2.6 ± 0.2	9.7 ± 4.8
	HTC Canadian Wonder raw			
		6.3 ± 0.3 <sup>a</sup>	1.8 ± 0.1 <sup>a</sup>	19.4 ± 9.7 <sup>a</sup>
	25% MC			
	140 °C	2.8 ± 0.3 <sup>b</sup>	1.0 ± 0.1 <sup>b</sup>	2.0 ± 0.1 <sup>b</sup>
	160 °C	2.2 ± 0.2 <sup>c</sup>	0.2 ± 0.0 <sup>c</sup>	nd
	180 °C	0.9 ± 0.1 <sup>d</sup>	nd	nd

<sup>a</sup> Dry matter. Different superscript letters within a column indicate statistically significant differences ( $p \leq 0.05$ ) for each type of bean cultivar. nd, not determined.

**Table 2. Effect of Extrusion Cooking on DF Content in HH and CW Beans<sup>a</sup>**

bean cultivar	extrusion conditions	IDF (%)	SDF (%)	TDF (%)	IDF/SDF
fresh Horsehead raw		15.5 ± 1.1 <sup>a</sup>	3.7 ± 0.3 <sup>a</sup>	19.2	4.2
	25% MC				
	140 °C	12.0 ± 0.8 <sup>b</sup>	5.7 ± 0.4 <sup>b</sup>	17.7	2.1
	160 °C	10.9 ± 0.8 <sup>b</sup>	6.2 ± 0.5 <sup>b</sup>	17.1	1.8
	170 °C	11.6 ± 0.9 <sup>b</sup>	6.6 ± 0.7 <sup>bc</sup>	18.2	1.8
	180 °C	10.8 ± 1.0 <sup>b</sup>	7.6 ± 0.6 <sup>c</sup>	18.4	1.4
	30% MC				
	140 °C	15.6 ± 1.2 <sup>a</sup>	5.8 ± 0.5 <sup>b</sup>	21.4	2.7
	160 °C	15.8 ± 1.3 <sup>a</sup>	6.9 ± 0.6 <sup>c</sup>	22.7	2.3
	170 °C	15.3 ± 1.1 <sup>a</sup>	8.6 ± 0.0 <sup>d</sup>	23.9	1.8
	180 °C	13.2 ± 0.9 <sup>b</sup>	9.2 ± 0.0 <sup>e</sup>	22.4	1.4
HTC Horsehead raw		15.3 ± 1.1 <sup>a</sup>	3.6 ± 0.3 <sup>a</sup>	18.9	4.2
	25% MC				
	170 °C	10.8 ± 0.8 <sup>b</sup>	7.4 ± 0.7 <sup>b</sup>	18.2	1.5
	180 °C	8.0 ± 0.6 <sup>c</sup>	8.2 ± 0.6 <sup>b</sup>	16.2	1.0
fresh Canadian Wonder raw		18.9 ± 1.4	4.5 ± 0.3	23.4	4.2
	HTC Canadian Wonder raw				
		19.7 ± 1.5 <sup>a</sup>	2.6 ± 0.2 <sup>a</sup>	22.3	7.5
	25% MC				
	140 °C	17.2 ± 1.3 <sup>a</sup>	3.5 ± 0.3 <sup>b</sup>	20.7	4.9
	160 °C	16.5 ± 1.2 <sup>b</sup>	4.5 ± 0.4 <sup>c</sup>	21.0	3.7
	180 °C	12.9 ± 1.0 <sup>c</sup>	4.9 ± 0.3 <sup>c</sup>	17.8	2.6

<sup>a</sup> Dry matter. Different superscript letters within a column indicate statistically significant differences ( $p \leq 0.05$ ) for each type of bean cultivar.

The levels of TDF in the fresh HH and CW beans as measured by using the AOAC procedure were 19.2 and 23.4 g/100 g of dm, respectively (Table 2), higher than in other important cereals and legumes (Bjorck et al., 1986; Gooneratne et al., 1994). Of this, >80% was in the form of IDF. The development of the HTC defect had no significant impact on these values. In fresh HH beans, the carbohydrate composition and Klason lignin content of IDF and SDF were also determined (Tables 3 and 4). In agreement with previously published data on legume seeds (Gooneratne et al., 1994), IDF of fresh

HH beans was mainly composed of glucose (35.6%), arabinose (29.0%), uronic acid (13.2%), and xylose (9.3%). The SDF consisted of neutral sugars, arabinose (33.3%) being the major contributor to this fraction followed by galactose/rhamnose (20.9%), indicating highly branched pectic polysaccharides. In keeping with the totals, the development of the HTC defect had little effect on these figures (Tables 5 and 6). The slightly higher levels of DF in CW (measured for the HTC form only) as compared with HH beans were due to higher levels of xylose and Klason lignin (Tables 5 and 6).

**Table 3. Monosaccharide Composition in Insoluble DF of Fresh HH Beans<sup>a</sup>**

extrusion conditions	composition of sugars						Klason lignin	IDF
	Glc	Xyl	Gal/Rha	Ara	Man	uronic acid		
raw	41.6 ± 3.1 <sup>a</sup>	10.9 ± 0.9 <sup>a</sup>	7.5 ± 0.6 <sup>a</sup>	33.9 ± 2.5 <sup>a</sup>	7.5 ± 0.6 <sup>a</sup>	15.4 ± 1.1 <sup>a</sup>	22.5 ± 2.0 <sup>a</sup>	139.3
extruded, 25% MC								
140 °C	35.0 ± 2.8 <sup>b</sup>	7.9 ± 0.5 <sup>b</sup>	4.9 ± 0.4 <sup>b</sup>	22.9 ± 1.8 <sup>b</sup>	5.2 ± 0.4 <sup>b</sup>	8.9 ± 0.7 <sup>b</sup>	15.0 ± 1.1 <sup>b</sup>	99.8
160 °C	27.5 ± 2.2 <sup>c</sup>	5.7 ± 0.4 <sup>c</sup>	2.7 ± 0.2 <sup>c</sup>	17.1 ± 1.3 <sup>c</sup>	4.8 ± 0.3 <sup>b</sup>	8.1 ± 0.6 <sup>b</sup>	27.1 ± 2.0 <sup>c</sup>	93.0
170 °C	21.2 ± 1.9 <sup>d</sup>	4.3 ± 0.3 <sup>d</sup>	2.2 ± 0.1 <sup>d</sup>	11.4 ± 0.8 <sup>d</sup>	4.5 ± 0.3 <sup>b</sup>	7.2 ± 0.6 <sup>c</sup>	14.4 ± 1.1 <sup>b</sup>	65.2
180 °C	33.1 ± 2.5 <sup>b</sup>	7.4 ± 0.6 <sup>b</sup>	3.6 ± 0.3 <sup>e</sup>	18.5 ± 1.4 <sup>e</sup>	4.3 ± 0.4 <sup>c</sup>	6.5 ± 0.4 <sup>c</sup>	24.5 ± 1.8 <sup>ac</sup>	97.9
extruded, 30% MC								
140 °C	41.9 ± 3.1 <sup>a</sup>	9.2 ± 0.8 <sup>a</sup>	5.0 ± 0.4 <sup>b</sup>	25.1 ± 1.9	6.4 ± 0.5 <sup>a</sup>	9.8 ± 0.7 <sup>b</sup>	27.0 ± 2.0 <sup>c</sup>	124.4
160 °C	26.4 ± 2.0 <sup>c</sup>	5.3 ± 0.4 <sup>c</sup>	1.8 ± 0.1 <sup>f</sup>	14.2 ± 1.3 <sup>e</sup>	nd	10.7 ± 0.8 <sup>d</sup>	21.4 ± 1.6 <sup>a</sup>	79.8
170 °C	27.6 ± 2.1 <sup>c</sup>	5.9 ± 0.4 <sup>c</sup>	2.9 ± 0.2 <sup>c</sup>	14.1 ± 1.1 <sup>e</sup>	nd	9.6 ± 0.7 <sup>b</sup>	22.8 ± 1.7 <sup>a</sup>	82.9
180 °C	24.7 ± 1.8 <sup>cd</sup>	6.5 ± 0.5 <sup>bc</sup>	5.8 ± 0.4 <sup>e</sup>	9.5 ± 0.8 <sup>f</sup>	nd	10.2 ± 0.8 <sup>b</sup>	26.5 ± 1.9 <sup>c</sup>	83.2

<sup>a</sup> Expressed as mg/g of dry matter. Different superscript letters within a column indicate statistically significant differences ( $p \leq 0.05$ ) for each type of bean cultivar. nd, not determined.

**Table 4. Monosaccharide Composition in Insoluble DF of HTC Beans<sup>a</sup>**

sample	extrusion conditions	composition of sugars						Klason lignin	IDF
		Glc	Xyl	Gal/Rha	Ara	Man	uronic acid		
HTC Horsehead									
raw		36.4 ± 2.9 <sup>a</sup>	9.9 ± 0.8 <sup>a</sup>	6.5 ± 0.5 <sup>a</sup>	36.8 ± 2.8 <sup>a</sup>	3.8 ± 0.3 <sup>a</sup>	12.2 ± 0.9 <sup>a</sup>	24.1 ± 2.5 <sup>a</sup>	129.7
	25% MC								
	170 °C	31.1 ± 2.3 <sup>b</sup>	5.9 ± 0.4 <sup>b</sup>	1.9 ± 0.1 <sup>b</sup>	19.7 ± 1.6 <sup>b</sup>	7.9 ± 0.6 <sup>b</sup>	6.6 ± 0.5 <sup>b</sup>	15.6 ± 1.6 <sup>b</sup>	88.7
	180 °C	24.8 ± 1.9 <sup>c</sup>	4.9 ± 0.4 <sup>c</sup>	1.8 ± 0.1 <sup>b</sup>	13.7 ± 1.0 <sup>c</sup>	5.8 ± 0.4 <sup>c</sup>	5.0 ± 0.4 <sup>c</sup>	14.0 ± 1.5 <sup>b</sup>	70.0
HTC Canadian Wonder									
raw		35.7 ± 2.7 <sup>a</sup>	12.1 ± 0.9 <sup>a</sup>	6.1 ± 0.5 <sup>a</sup>	37.5 ± 2.6 <sup>a</sup>	4.7 ± 0.4 <sup>a</sup>	11.5 ± 1.0 <sup>a</sup>	28.7 ± 2.9 <sup>a</sup>	136.3
	25% MC								
	140 °C	44.2 ± 3.3 <sup>b</sup>	13.9 ± 1.0 <sup>a</sup>	7.3 ± 0.5 <sup>b</sup>	37.5 ± 2.9 <sup>a</sup>	8.6 ± 0.6 <sup>b</sup>	13.4 ± 1.0 <sup>a</sup>	26.0 ± 2.9 <sup>a</sup>	150.9
	160 °C	46.6 ± 3.7 <sup>b</sup>	11.1 ± 0.9 <sup>a</sup>	5.1 ± 0.4 <sup>c</sup>	34.9 ± 3.2 <sup>a</sup>	4.9 ± 0.4 <sup>a</sup>	12.0 ± 0.9 <sup>a</sup>	28.1 ± 2.8 <sup>a</sup>	142.7
	180 °C	36.9 ± 2.8 <sup>a</sup>	9.9 ± 0.7 <sup>b</sup>	3.7 ± 0.3 <sup>d</sup>	27.2 ± 2.0 <sup>b</sup>	3.3 ± 0.3 <sup>c</sup>	7.9 ± 0.6 <sup>b</sup>	16.8 ± 1.8 <sup>b</sup>	105.7

<sup>a</sup> Expressed as mg/g of dry matter. Different superscript letters within a column indicate statistically significant differences ( $p \leq 0.05$ ) for each type of bean cultivar.

**Table 5. Monosaccharide Composition in Soluble DF of Fresh HH Beans<sup>a</sup>**

extrusion conditions	composition of sugars						SDF
	Glc	Xyl	Gal/Rha	Ara	Man	uronic acid	
raw	2.5 ± 0.2 <sup>a</sup>	1.8 ± 0.1 <sup>a</sup>	4.4 ± 0.3 <sup>a</sup>	7.0 ± 0.5 <sup>a</sup>	3.3 ± 0.2 <sup>a</sup>	2.1 ± 0.2 <sup>a</sup>	21.1
extruded, 25% MC							
140 °C	2.1 ± 0.2 <sup>a</sup>	5.1 ± 0.4 <sup>b</sup>	6.2 ± 0.5 <sup>b</sup>	16.8 ± 1.2 <sup>b</sup>	2.5 ± 0.2 <sup>b</sup>	3.8 ± 0.3 <sup>b</sup>	36.5
160 °C	1.7 ± 0.1 <sup>b</sup>	3.3 ± 0.2 <sup>c</sup>	4.5 ± 0.3 <sup>a</sup>	12.3 ± 0.9 <sup>c</sup>	1.8 ± 0.1 <sup>c</sup>	4.8 ± 0.4 <sup>c</sup>	28.4
170 °C	2.4 ± 0.2 <sup>a</sup>	3.4 ± 0.2 <sup>c</sup>	5.2 ± 0.4 <sup>c</sup>	11.4 ± 0.8 <sup>c</sup>	1.9 ± 0.1 <sup>c</sup>	5.3 ± 0.4 <sup>c</sup>	29.6
180 °C	2.5 ± 0.2 <sup>a</sup>	4.3 ± 0.4 <sup>b</sup>	6.1 ± 0.5 <sup>bc</sup>	18.3 ± 1.4 <sup>b</sup>	1.8 ± 0.1 <sup>c</sup>	5.9 ± 0.5 <sup>d</sup>	38.9
extruded, 30% MC							
140 °C	2.3 ± 0.2 <sup>a</sup>	5.1 ± 0.4 <sup>b</sup>	6.1 ± 0.5 <sup>bc</sup>	13.3 ± 1.0 <sup>c</sup>	2.3 ± 0.2 <sup>b</sup>	4.5 ± 0.4 <sup>bc</sup>	33.6
160 °C	1.7 ± 0.1 <sup>b</sup>	3.7 ± 0.3 <sup>c</sup>	4.7 ± 0.4 <sup>ac</sup>	10.4 ± 0.9 <sup>d</sup>	1.1 ± 0.1 <sup>d</sup>	6.2 ± 0.5 <sup>d</sup>	27.8
170 °C	2.7 ± 0.2 <sup>a</sup>	3.4 ± 0.2 <sup>c</sup>	4.8 ± 0.4 <sup>ac</sup>	10.3 ± 0.8 <sup>d</sup>	1.1 ± 0.1 <sup>d</sup>	7.4 ± 0.6 <sup>e</sup>	29.7
180 °C	1.3 ± 0.1 <sup>c</sup>	3.7 ± 0.3 <sup>c</sup>	4.7 ± 0.3 <sup>ac</sup>	12.5 ± 0.9 <sup>c</sup>	1.5 ± 0.1 <sup>e</sup>	7.4 ± 0.6 <sup>e</sup>	31.1

<sup>a</sup> Expressed as mg/g of dry matter. Different superscript letters within a column indicate statistically significant differences ( $p \leq 0.05$ ) for each type of bean cultivar.

**Effect of Extrusion on Physical Properties.** Initial extrusion studies showed that screw speed had little effect on extrudate products, barrel temperature being the key variable (results not shown). Regarding the physical properties of bean flours, bulk density data (Figure 1) of the extrudates of fresh HH flours at different moisture contents and temperatures revealed that thermal energy input is dominant, and the density falls with increasing barrel temperature (64–56% reduction at 25 and 30% MC, respectively) as observed in most cases with extrusion of bean flour (Edwards et al., 1994), maize (Fletcher et al., 1985), and soy protein (Cumming et al., 1972). Similar bulk density changes with increased barrel temperature were observed in extrudates from fresh CW bean flour with 30% MC; at 140 °C the extrudate was dense (Figure 2a), whereas at 180 °C the extrudate contained air bubbles and was of greater diameter (Figure 2b). HTC CW beans showed the same trend, although the bulk density decreased to

a lesser extent (49%) (Figure 1). The bulk density was  $1.4 \pm 0.2 \text{ g mL}^{-1}$  for specific mechanical energies of 6–10  $\text{W} \cdot \text{min g}^{-1}$ , which contrasts with the greater variation reported by Edwards et al. (1994). The bulk density values are not as low as those of highly expanded cereal extrudates, for which extrusion conditions of low moisture and high temperature lead to high mechanical energy dissipation, microstructural degradation, and consequent high expansion (Kirby et al., 1988). The moisture contents of the bean flours used in this study have no significant effect on the extrudate density. Generally, however, die size, moisture content, extrusion temperature, screw speed, and feed rate affect expansion and bulk density of starchy materials as described more fully elsewhere (Fletcher et al., 1985; Colonna et al., 1989).

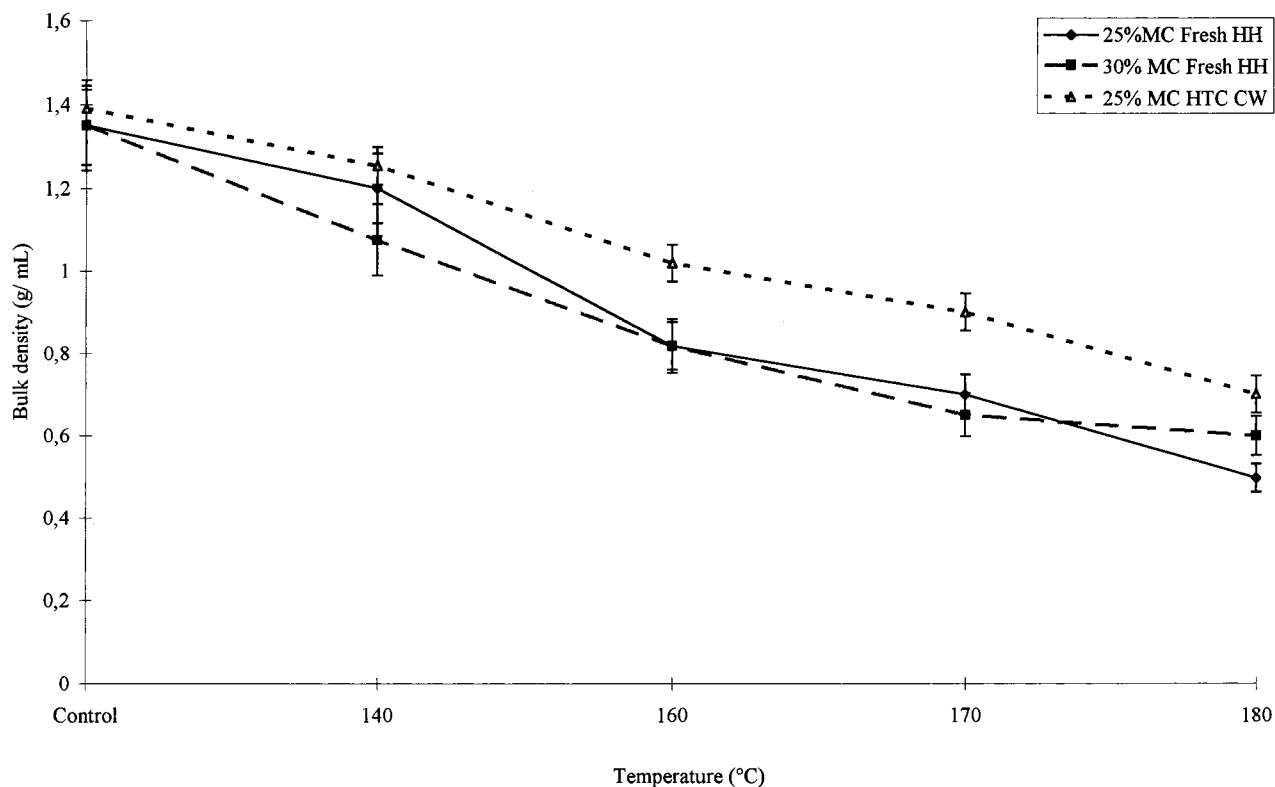
Some functional properties of the extrusion-cooked bean flours are presented in Figure 3. The WSI and WAI values are comparable with those for maize extrudates



**Table 6. Monosaccharide Composition in Soluble DF of HTC Beans<sup>a</sup>**

sample	extrusion conditions	composition of sugars						SDF
		Glc	Xyl	Gal/Rha	Ara	Man	uronic acid	
HTC Horsehead raw		3.3 ± 0.3 <sup>a</sup>	2.5 ± 0.2 <sup>a</sup>	5.5 ± 0.4 <sup>a</sup>	7.3 ± 0.7 <sup>a</sup>	1.7 ± 0.2 <sup>a</sup>	4.3 ± 0.5 <sup>a</sup>	24.6
	25% MC							
	170 °C	3.2 ± 0.3 <sup>a</sup>	5.3 ± 0.4 <sup>b</sup>	6.4 ± 0.5 <sup>a</sup>	18.9 ± 1.9 <sup>b</sup>	3.3 ± 0.3 <sup>b</sup>	6.7 ± 0.6 <sup>b</sup>	43.8
	180 °C	2.4 ± 0.2 <sup>b</sup>	4.5 ± 0.4 <sup>b</sup>	6.8 ± 0.6 <sup>b</sup>	20.5 ± 2.1 <sup>b</sup>	2.0 ± 0.2 <sup>a</sup>	5.5 ± 0.5 <sup>c</sup>	41.7
HTC Canadian Wonder raw		1.6 ± 0.1 <sup>a</sup>	0.9 ± 0.1 <sup>a</sup>	2.6 ± 0.2 <sup>a</sup>	2.9 ± 0.3 <sup>a</sup>	2.9 ± 0.2 <sup>a</sup>	1.3 ± 0.1 <sup>a</sup>	12.2
	25% MC							
	140 °C	2.6 ± 0.2 <sup>b</sup>	2.0 ± 0.2 <sup>b</sup>	4.5 ± 0.3 <sup>b</sup>	6.7 ± 0.6 <sup>b</sup>	3.0 ± 0.3 <sup>a</sup>	3.9 ± 0.4 <sup>b</sup>	22.7
	160 °C	2.1 ± 0.1 <sup>b</sup>	2.4 ± 0.2 <sup>b</sup>	4.4 ± 0.3 <sup>b</sup>	8.1 ± 0.8 <sup>b</sup>	2.9 ± 0.2 <sup>a</sup>	4.1 ± 0.4 <sup>bc</sup>	24.0
	180 °C	1.9 ± 0.1 <sup>c</sup>	2.6 ± 0.2 <sup>c</sup>	5.0 ± 0.4 <sup>b</sup>	11.0 ± 1.1 <sup>c</sup>	2.5 ± 0.2 <sup>a</sup>	4.9 ± 0.4 <sup>c</sup>	27.9

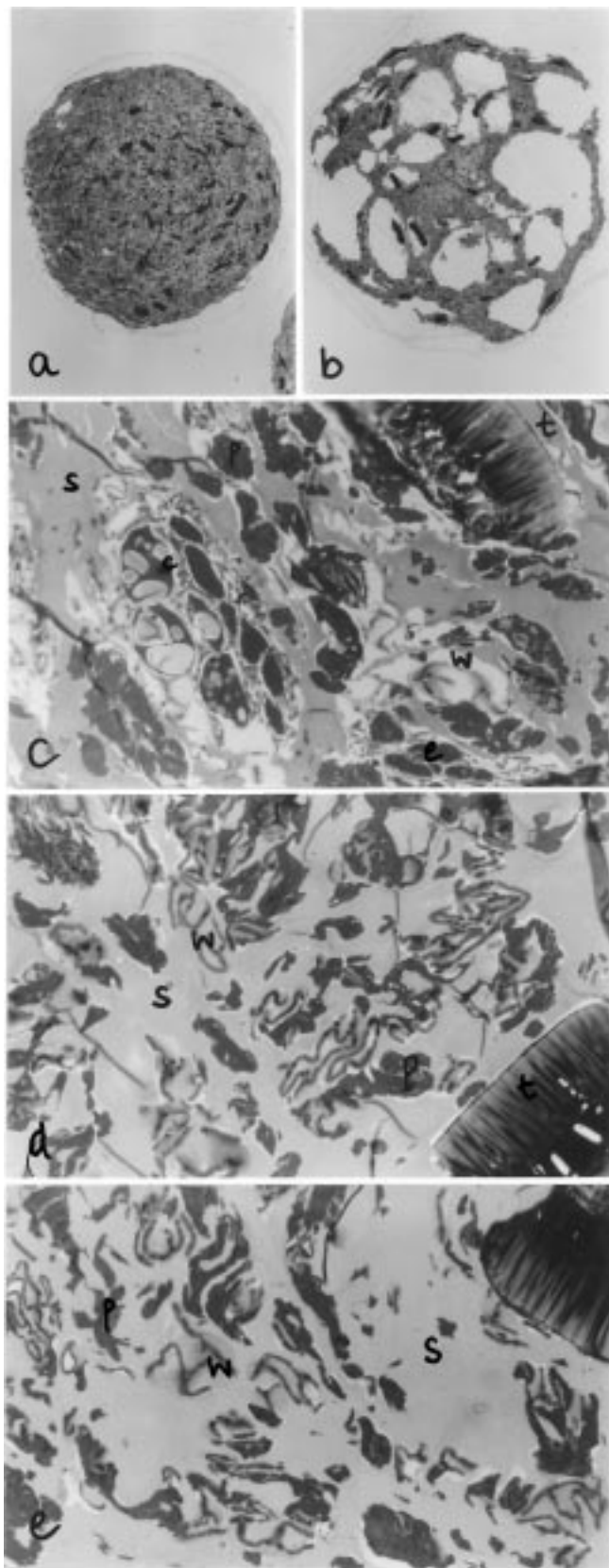
<sup>a</sup> Expressed as mg/g of dry matter. Different superscript letters within a column indicate statistically significant differences ( $p \leq 0.05$ ) for each type of bean cultivar.

**Figure 1.** Effect of extrusion cooking on bulk density of bean flours.

(Harper, 1981; Mercier and Feillet, 1975; Kirby et al., 1988) and for extruded soybean (Cumming et al., 1972). They are, however, lower than the values for extruded bean flour given by Steel et al. (1995). The increase in WAI with increasing temperature (53%) is probably due to the higher proportion of gelatinized starch in the extruded samples. Similar results were described by Pilosof et al. (1982), Han and Khan (1990), and Avin et al. (1992) in bean and pea flours and for soybeans (Aguilera and Kosikowski, 1976; Cumming et al., 1972). In contrast, the WSI decreased with increasing barrel temperature (23%). Most published data for WSI are for high-starch-containing cereals, and the WSI increases with increasing temperature (Mercier and Feillet, 1975; Kirby et al., 1988). However, protein denaturation is known to decrease protein solubility as shown by a decrease in nitrogen-solubility index with increasing temperature for extrusion of beans (Pham and Del Rosario, 1984); hence, the results here appear to exhibit characteristics of both systems and may influence protein digestibility.

**Microstructure of Extrudates.** All extrudates contained recognizable fragments of the principal tissues of beans. Sections of extruded fresh CW bean flour of 25% MC that had been subjected to barrel temperatures of 140, 160, and 180 °C are illustrated (Figure 2c–e). At 140 °C (Figure 2c), most of the extrudate consisted of testa fragments (t), cotyledon cell contents rich in storage proteins (p), slightly swollen cotyledon cell walls (w) with dark-staining middle lamella, embryo tissue (e), and groups of whole cotyledon cells (c) containing starch granules, embedded in a matrix of gelatinized starch (s). In some regions toward the center of the extrudate the starch was only partially gelatinized (not shown).

At 160 °C, gelatinization of the starch matrix (s) was complete (Figure 2d). Regions of swollen cell wall material (w) were present, but near the surface of the extrudate some wall material had been extracted, presumably in the aqueous solutions used in the embedding procedure. Protein-rich cell contents (p) retained a rounded profile. At 180 °C (Figure 2e), much



**Figure 2.** Transverse sections of extrusions of fresh Canadian Wonder bean flour (a) 140 °C and 30% MC,  $\times 9$ , (b) 180 °C and 30% MC,  $\times 9$ , (c) 140 °C and 25% MC,  $\times 250$ , (d) 160 °C and 25% MC,  $\times 250$ , and (e) 180 °C and 25% MC,  $\times 250$ . c, whole cotyledon cells; e, embryo tissue; p, protein-rich cotyledon cell contents; s, gelatinized starch; t, testa; w, cotyledon cell walls.

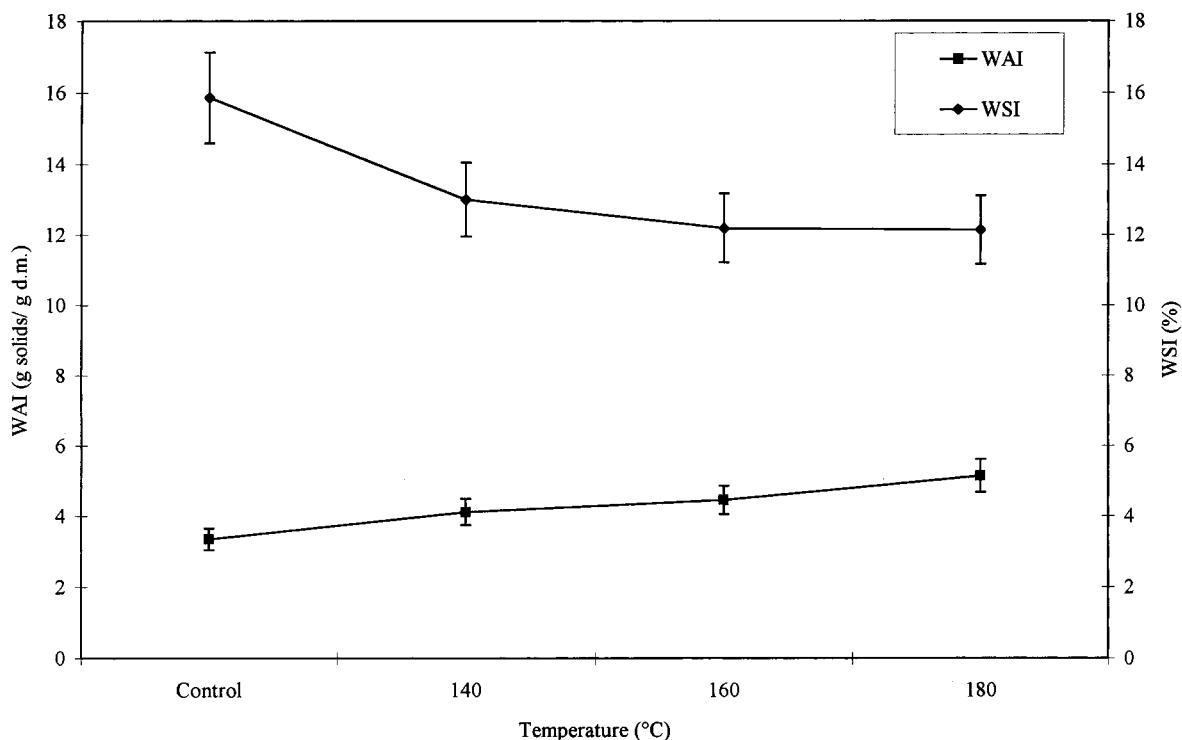
of the swollen cotyledon cell wall material (w) was

extracted with only the dark-staining middle lamella remaining (Figure 2e). Protein-rich regions (p) were deformed at the highest barrel temperature, but the structure of the testa (t) appeared to be unaffected.

**Extrusion-Induced Inactivation of Antinutrients.** Extrusion cooking was efficient in the inactivation of bean antinutrients, depending on barrel temperature (Table 1). Lectin activity falls dramatically at 140 °C and above in both bean cultivars; below this temperature, it might not be fully inactivated. Steel et al. (1995) reported 98.5% lectin inactivation at a high extrusion temperature (190 °C) in beans.  $\alpha$ -Amylase inhibitor was not detected in any extruded fresh or HTC HH beans, but a significant residual activity (55%) was found at 140 °C in HTC CW beans. In contrast, trypsin inhibitor was the antinutrient that exhibited more resistance to extrusion, although the increase of temperature markedly reduced inhibitor activity, which was no longer detected at 180 °C. Similar decreases in trypsin inhibitor with increasing temperature were found by Aguilera and Kosikowski (1976) for soybean and by Edwards et al. (1994) and Steel et al. (1995) for bean flours. Complete inactivation of the trypsin inhibitors is difficult to accomplish, and a residual activity is found even at 180 °C (25%) in very HTC CW beans, comparable to the levels normally found in processed foods. The inactivation was accentuated at the lower moisture (25% MC) for fresh HH beans. Moisture is a well-known essential factor in the destruction or inactivation of enzyme inhibitors, particularly trypsin inhibitors in soybeans and legumes (Phillips et al., 1983; Buera et al., 1984), and its role is more complex in extrusion (Aguilera and Kosikowski, 1976; Edwards et al., 1994).

**Extrusion-Induced Modifications of DF.** The use of single-screw extrusion also caused important changes in other nutritional components of beans such as the DF fractions. Extrusion cooking of fresh HH caused decreases in IDF from 15.5 to 10.8% dm (Table 2), being greater at 25% MC in agreement with Camire et al. (1997). The resistant protein, present in the IDF residues, also decreased (13–44%, data not shown) due to the increase in protein digestibility. In contrast, a significant increase of SDF was found in extruded beans, 9.2 versus 3.7% dm for extruded and raw common beans, respectively. The extent of solubilization depended upon the extrusion conditions; the greatest increase of SDF appeared with 30% MC. Thus, although the level of TDF in HH beans was not affected by extrusion, a significant redistribution of insoluble to more water-soluble fiber fractions took place, the extent of which depended on temperature and MC. Thus, in raw HH bean flour, 19% of total fiber was soluble versus 27–43% for extruded products. Similar trends were also found for CW beans during the extrusion process (Table 2). These results are in agreement with those of Lue et al. (1991), who reported decreased insoluble fiber with no net change in TDF content after extrusion of cornmeal and sugar beet fiber mixtures, and those of Bjorck et al. (1986); Siljestrom et al. (1986) in whole-grain wheat flour; and Fornal et al. (1987) in extruded buckwheat and barley. In addition, HTC samples showed a similar trend to fresh counterparts.

HPLC analysis of DF sugar constituents confirmed the solubilization observed in the gravimetric assay. Extrusion cooking in HH beans produced an increased solubilization of insoluble fiber compounds, especially arabinose (30–66%), uronic acid (42–58%), and glucose



**Figure 3.** Effects of extrusion cooking (25% MC) on WAI and WSI of HTC Canadian Wonder beans.

(15–48%) (Table 3). A significant degradation and solubilization of pectic polysaccharides and cellulose was observed. Although high MC (30%) generally induced more sugar losses with the exception of uronic acids and galactose/rhamnose, barrel temperature was the key element of extrusion and caused the greatest changes, in agreement with the findings of Camire et al. (1997). A similar tendency was observed in HTC bean flours (Table 4), with a more accentuated redistribution of DF fractions in induced HTC HH samples (29–49% IDF reduction in HTC HH beans and 12.7–34.5% in HTC CW beans). Klason lignin data were not conclusive. Similar values were exhibited in fresh HH (Table 3), and significant decreases were shown in HTC beans (Table 4). However, Ralet et al. (1990) found increases of lignin in wheat bran extruded under conditions leading to high specific mechanical energy. This increase could be due to the physicochemical modifications of carbohydrates and other compounds into new components that may be resistant to enzymatic digestion and acid hydrolysis. Because the Klason lignin procedure does not identify true lignin, more research is needed to define and determine any newly formed materials.

Regarding the SDF fraction in fresh and HTC beans, neutral sugars (arabinose) were enhanced by lower temperatures (78–279%), whereas uronic acids were enhanced by higher temperatures (28–277%) (Tables 5 and 6). These results indicate that extrusion cooking leads to the solubilization of neutral pectic substances or pectic side chains, rather than homogalacturonans or hemicellulosic polymers, and also that the cellulosic backbone was not degraded. The branched structure of pectin may be more susceptible to shear during extrusion (Ralet et al., 1993; Gooneratne et al., 1994). Furthermore, Gourgue et al. (1994) reported significant increases in water-soluble uronic acids associated with increased viscosities after extrusion of orange and lemon peels. However, no changes in uronic acids after extrusion have been observed for wheat flour (Theander and

Westerlund, 1987), cornmeal, oatmeal, and potato peels (Camire and Flint, 1991). In cereals, uronic acid derives from hemicelluloses, not pectins, whereas in beans, pectins are the primary source of acid sugars (Gooneratne et al., 1994).

#### CONCLUSIONS

(1) Single-screw extrusion of the fresh and HTC bean flour resulted in a decreased extrudate bulk density, a decreased WSI with increasing temperature, and an increase in WAI due to the higher proportion of gelatinized starch in the extruded samples.

(2) Both fresh and HTC beans contained nutritionally significant amounts of lectins, trypsin, and  $\alpha$ -amylase inhibitors, which were mostly inactivated by extrusion. Extrusion therefore has the potential to produce non-toxic flour from HTC beans for cooked and directly consumed products.

(3) Extrusion also caused a considerable solubilization of DF components, particularly pectic polymers, although the TDF content was not affected.

(4) Generally, extrudates from HTC beans showed physical and chemical characteristics similar to those of extrudates from fresh beans.

#### ACKNOWLEDGMENT

We thank Dr. B. Clark (Cranfield University, Silsoe College) for the use of the Brabender extruder.

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Received for review July 31, 1998. Revised manuscript received November 30, 1998. Accepted December 14, 1998. We thank the European Communities for their financial support (STD-3 Project TS3-CT92-0085). This work was also partly financed by the U.K. Office of Science and Technology (M.L.P., A.C.S., and K.W.W.).

JF980850M