SODIUM BICARBONATE AND THE MICROSTRUCTURE, EXPANSION AND COLOR OF EXTRUDED BLACK BEANS

JOSE DE J. BERRIOS1, DELILAH F. WOOD2, LINDA WHITEHAND and JAMES PAN

U.S. Department of Agriculture
Agriculture Research Service
Western Regional Research Center (WRRC)
800 Buchanan Street
Albany, CA 94710-1105

Accepted for Publication October 29, 2004

ABSTRACT

Seed microstructure provides complementary information to physico-chemical determinations. Scanning electron micrographs of cross sections of black bean extrudates illustrated the increase in volume expansion, with an increase of sodium bicarbonate (NaHCO₃) in the extrudate. This increase in volume expansion is attributed to the increase in air cell size and corresponding decrease in air cell wall thickness with additional concentrations of NaHCO₃. The measured increase in diameter and expansion ratio of extrudates with NaHCO₃ addition may be explained by the increase in number of air cells within the extrudate and the increase in pores in the gelatinized starch matrix of air cell walls. Extrusion conditions, which involved the use of heat and moisture, provided the necessary conditions for the release of CO₂ from NaHCO₃ during processing. Expansion ratio increases between the control extrudate, and extrudate with 0.5% NaHCO₃ addition were twofold at the node and 1.8-fold at the area between the nodes. Statistical analysis of color data exhibited no significant change in L*, hue or chroma across concentrations of NaHCO₃ for nonextruded flours. A simple linear regression adequately described changes in L* and hue for extruded flours. However, a curvilinear relationship was needed to explain changes in chroma versus NaHCO₃ for extruded flours, making changes in chroma measurements less easy to interpret. The color data in this study serve as primary information for future establishment of cut-off values of color for the development of an acceptable legume snack.

1 Corresponding author. TEL: (510) 559-5652; FAX: (510) 559-5820; EMAIL: jberrios@mail.pw.usda.gov
2 Formerly published as Delilah W. Irving.
INTRODUCTION

Microstructural studies using scanning electron microscopy (SEM), transmission electron microscopy and light microscopy (LM) techniques have been applied to raw and processed legumes by several researchers (Hahn et al. 1977; Ben-Hdech et al. 1991; Garcia et al. 1993; Gujska et al. 1994; Berrios et al. 1998) to obtain structure–function information that can then be related to physicochemical determinations. Wood et al. (1998), using SEM and LM, observed that the initial raw states of starch and protein components in black bean purée were greatly modified in the final drum-dried product. Ingredients such as leavening agents used as food processing aids may induce changes in microstructure as well. Sodium bicarbonate (NaHCO₃) is a leavening agent commonly used in the bakery industry, and more recently it has been used in directly expanded extruded cereal products to enhance product physical properties and acceptability (Lai et al. 1989; Lajoie et al. 1996; Parson et al. 1996). More recently, NaHCO₃ was used to produce expanded snack-type products by twin-screw extrusion processing (Berrios et al. 2002).

Color is an important component of the visual appearance of foods and nonfood materials. Additionally, color may also be related to the wholesomeness of foods. Along with flavor and texture, color is often perceived as a valuable quality factor in the acceptability and marketability of food products. Color is perceived three dimensionally, based on responses of three different receptors (red, green and blue) in the human eye (Francis and Clydesdale 1975), yet all three dimensions may not be of practical importance (Francis 1995). The Judd–Hunter L, a, b and CIELAB L*, a*, b* are alternative color scales used to measure the degree of lightness (L*), redness or greenness (+a*) and yellowness or blueness (+b*), with the CIELAB scale being most commonly used for the evaluation of color in foods (CIE 1986). Conversion of a* and b* readings to hue and chroma values gives results more closely associated with human perception (Setser 1984). The purpose of this study was to evaluate the effect of adding selected concentrations of sodium bicarbonate to black bean flours on the structure, expansion and color characteristics of black bean extrudates.

MATERIALS AND METHODS

Commercial black beans (Phaseolus vulgaris L.) were blended in a paddle-type mixer (Marion Mixer, Rapid Machinery Co., Marion, IA) to a uniform lot and ground in a disc attrition mill (CE-Bouer, Springfield, OH) equipped with a Dayton AC inverter model no. 1XC98A (Dayton Electric Mfg. Co., IL) operated at 3600 rpm, using 8118 plates. Proximate analysis
showed that the black bean flour used in this study contained 9.83% moisture, 24.68% crude protein, 2.25% crude fat, 4.30% ash and 58.94% total carbohydrates (calculated by difference). Certified A.C.S. NaHCO₃ (Fisher Scientific, Fair Lawn, NJ) was added to bean flours in selected proportions to yield flours containing 0.0, 0.1, 0.2, 0.3, 0.4, or 0.5% of NaHCO₃ and mixed in a Hobart mixer (Model A-200, The Hobart Mfg. Co., Troy, OH) for 5 min at speed 1 before the flour–NaHCO₃ mixtures were extruded.

Extruder and Processing Conditions

A Leistritz Micro-18-GL twin-screw extruder (American Leistritz Extruder Corporation, Sommerville, NJ) equipped with a 5 hp motor and corotating intermeshing screws containing three pairs of right-handed kneading blocks were used at a constant screw speed of 200 rpm. Additional extruder parameters were previously described by Berrios et al. (2002). The flour mixtures were fed into the extruder at a rate of 80 g/min and extruded at moisture content of 20% (wwb) by adjusting the amount of water in the extruder. Extruded bean rods were collected in stainless steel trays for 6 min, starting approximately 10 min after the operation conditions of torque and pressure reached steady state. Extruded bean rods were cooled to room temperature. The diameter of extrudate was measured and the extrudate packaged in zip-lock plastic bags. After bulk density determination, a set of bean extrudates was refrigerated for microscopic evaluation, and another set was ground for color measurement.

Scanning Electron Microscopy

Extruded bean flours in the form of rods were fractured with a razor blade and mounted onto aluminum specimen stubs using carbon conductive tabs and sputter coated with gold–palladium in a Denton Desk II sputter coating unit. The extrudates were observed and photographed in a Hitachi S-530 scanning electron microscope (SEM) or a Hitachi S-4700 Field Emission SEM (Hitachi Instruments Service Co., Ltd., Tokyo, Japan) at ambient temperature and 1 to 5 kV. Images were recorded by using Kodak T-Max 400 4” × 5” sheet film and converted to digital files, or were recorded digitally.

Light Microscopy

Sections of the bean flour extrudates were prepared by fixing blocks of 2 mm³ of the extrudate in a fixative containing 3% glutaraldehyde, 2% formaldehyde and ethanol–cacodylate buffer (50% ethanol and 0.05 M cacodylate), pH 6.9, overnight at 4C. Fixed extrudates were rinsed in ethanol–cacodylate buffer for 10 min. The rinse was repeated three times. The extrudates were
dehydrated in an ethanol graded series (70%, 95% and 3 \times 100%, 20 min each) and infiltrated with glycol methacrylate (GMA, Technovit 7100, Germany) and catalyst at 21°C. The dehydrated extrudates were flat embedded and sectioned to a 2–4 \mu m thickness by using glass knives with a Sorvall Porter-Blum MT-2 ultramicrotome (Diversified Equipment Co., Inc., Lorton, VA).

Sections were stained for starch and proteins simultaneously by treating sections for 15 min in a Coplin staining jar containing 0.5% aqueous Safranin O. Following staining, slides were rinsed in running tap water, air dried and mounted in immersion oil. Sections were viewed and photographed by using a fluorescence filter set at a 450–490 nm excitation wavelength. Under these conditions, starch fluoresces yellow-green and proteins appear pink to red. As a second verification for proteins, sections were stained for 2 min in 0.01% acid fuchsin in 1.0% acetic acid, rinsed in running tap water, air dried and mounted in immersion oil (Fulcher and Wong 1980). Under this condition, proteins appear red when viewed under fluorescence illumination at a 546 nm excitation wavelength. For determination of starch granules and amylose, sections were stained with Iodine/potassium Iodide (I$_2$KI) (Clark 1991), rinsed briefly in running tap water and mounted in water. Ungelatinized starch granules were detected by mounting unstained sections in immersion oil and viewing the sections through crossed polarizing lenses.

**Extrudate Diameter, Expansion Ratio and Bulk Density**

Cross-section diameters of black bean extrudates were measured randomly with a digital caliper in millimeters at two different places, at the node and between the nodes. A total of 20 measurements were made per treatment, and the expansion ratio of the extrudates, in the form of rods, was calculated by dividing the cross-sectional area of the extrudates by the cross-sectional area of the 2 mm die orifice. Mean expansion ratios are reported. Bulk density was determined in triplicate with a sand displacement method as described by Edwards et al. (1994).

**Color Evaluation**

Nonextruded black beans and black bean extrudates were milled in a Udy mill (Udy Corporation, Fort Collins, CO) through a 0.5 mm screen before color evaluation. The colorimeter, a Minolta Chroma-Meter CR-200 (Minolta Corporation, Ramsey, NJ) equipped with a xenon lamp in the L*, a* and b* system, was calibrated with a white tile (L* = 94.4000; a* = 0.3134; b* = 0.3205). Color measurements were determined by holding the sensing head in direct contact with the surface of the flours. Triplicate measurements were determined at random surface locations on each flour. The L*, a* and b* readings obtained directly from the instrument provide measures of light-
ness, redness and yellowness, respectively. These readings were converted to the $L^*C^*H^*$ system by using $a^*$ and $b^*$ to calculate values of hue ($h_{ab}$), a description of the color, and chroma ($C_{ab}$), a measure of color saturation (vividness or dullness). Hue, the angle expressed in degrees from pure red at $0^\circ$, to yellow at $90^\circ$, green at $180^\circ$ and blue at $270^\circ$, and chroma, the distance from the center of color space, were calculated as follows (X-Rite 1993):

$$
\text{Hue is } h_{ab} = \arctan\left(\frac{b^*}{a^*}\right) \text{ or } \tan^{-1}\left(\frac{b^*}{a^*}\right), \text{ whereas }
$$

$$
\text{Chroma is } C_{ab} = (a^{*2} + b^{*2})^{1/2}
$$

**Statistical Analysis**

For statistical analysis of color results, means of triplicate measurements of $L^*$, hue and chroma at each concentration of NaHCO$_3$ for nonextruded and extruded bean flours were fit with a general linear model using the SAS GLM procedure (SAS 1989). The model was chosen to provide estimates and test statistics for the overall difference between extruded material and the nonextruded control (discrete effect for treatment), the change across concentrations of NaHCO$_3$ (regression – linear or curvilinear) and, perhaps most importantly, the difference between the regression for extruded and for nonextruded flour (interaction). For ease of interpretation, results are presented graphically with prediction lines and 95% confidence intervals. For this purpose, the SAS/GRAPH Software Version 6 (SAS 1990) was used.

**RESULTS AND DISCUSSION**

**Microstructural Evaluation**

Scanning electron microscopy observation of cross sections of black bean control extrudates exhibited the presence of small numbers of air cells of irregular size (Fig. 1a). Also, the control extrudate exhibited the smallest overall expansion when compared to extrudates with added NaHCO$_3$. Entire cross sections of extrudates are presented at equivalent magnifications of 1 mm to illustrate changes in air cell dimensions (Fig. 1a–f). Addition of NaHCO$_3$ decreased the mean air cell size and promoted air cell size uniformity. Moreover, the number of air cells increased and cell wall thickness decreased with an increase in NaHCO$_3$ concentration. The combined effect of these two development factors may result in the collapse of cell walls and the appearance of large void spaces within the extrudates. The collapse of cell walls and the appearance of large void spaces within the extrudates was observed as the expansion volume of the extrudate increased with an increase in NaHCO$_3$ concentration (Fig. 1b–f). Similarly, Lai et al. (1989) reported that
the addition of sodium bicarbonate or sodium carbonate to wheat starch improved expansion but weakened the structure of wheat starch extrudates.

The air cell walls of black bean extrudates are composed mainly of gelatinized starch matrices, cooked protein inclusions (Fig. 2), cotyledon bean
cell wall components (Fig. 2c), intact cells containing partly gelatinized starch granules and protein (Fig. 2d,e), and seed coat fragments (Figs. 2a and 3c). Most cellular components are readily identified in SEM micrographs by correlating the shapes to the stained components viewed by light microscopy. The starch matrices expand (Figs. 2a,f and 3), and protein networks become smoother and more elongated with increasing concentrations of NaHCO₃ (Fig. 2). Air cell walls containing heat-denatured proteins (Fig. 2b,f) and seed coat fragments embedded in the gelatinized starch matrix were observed (Figs. 2a and 4c,d). Seed coat fragments embedded in the gelatinized starch matrices were also observed in drum-dried black bean flakes by Wood et al. (1998), indicating that thermal processing did not destroy seed coat fragments.

Large air gaps occurred at the junctions of the seed coat and starch matrix constituents (Fig. 3c), and smaller air gaps were observed among proteins and starch matrices (Fig. 3). The numbers of small air cells and smaller pores (tiny air cells) increased with increasing concentrations of NaHCO₃. The relative amounts of space occupied by the starch matrices, as indicated by fewer protein inclusions in a field of view, also increased with increasing NaHCO₃ concentrations (Fig. 4). The tiny air cells were more evident in the gelatinized starch matrices at high magnification and also observed in the cooked protein inclusions (Fig. 5a,b,c,e). However, these tiny air cells were less abundant than those in the gelatinized starch matrices. Fractured protein inclusions containing pores with jagged edges were attributed to fracturing that occurred during preparation of extrudates for SEM. Protein bodies contain crystalline inclusions of phytic acid that were apparently retained in the protein associations after extrusion. The hard crystals of phytic acid may be responsible for the jagged edged pores in the protein associations (Fig. 5d).

**NaHCO₃ and Diameter, Expansion Ratio and Bulk Density**

Expansion of the extrudates was evident when the molten bean mixture was expelled under high pressure through the extruder restriction dies (2 mm
diameter) in the form of rods. The rapid reduction in pressure resulted in intensive flash-off of internal moisture and the subsequent expansion of the extrudate. Guy and Horne (1988) reported that the elastic character of the molten extrudate creates a die swell, which controls the overall expansion of the extrudate.

Temperature of the molten bean extrudate was determined at the dies before measurement of extrudate rod diameter. The extruded material close to the die was too hot and too soft for diameter measurement. Therefore, the

FIG. 4. SCANNING ELECTRON MICROGRAPHS EXHIBITING AIR CELL WALLS OF BLACK BEAN EXTRUDATES WITH ADDED NaHCO₃ AT (a) 0%, CONTROL; (b) 0.1%; (c) 0.2%; (d) 0.3%; (e) 0.4%; AND (f) 0.5%

The numbers of small air cells (Ac) and small pores (Po) increase with increasing concentrations of NaHCO₃. Other cellular components observed are: starch matrix (Sm), protein (P) and cell wall (cw). Magnification bar = 20 μm.
extruded material was cooled to room temperature before the diameter was determined. The diameter of the extrudate decreased slightly upon cooling and setting of the extrudate structure. Mercier et al. (1989) also reported that as the temperature of the extruded melt decreased, the extrudate solidifies and retains its expanded shape. Carbon dioxide is produced during heating (Ben- nion 1980) or dissolving of NaHCO₃ in aqueous media (Anonymous 1983) according to the following reaction:

\[
2 \text{NaHCO}_3 \xrightarrow{\text{Heat} \, + \, \text{Water}} \text{Na}_2\text{CO}_3 + \text{CO}_2 + \text{H}_2\text{O}.
\]

The conversion is 100% at 100°C. As the bean flour–NaHCO₃ mixtures were heated at 20% moisture during extrusion cooking, production of CO₂ and a subsequent increase in expansion of the extrudates was expected. The increase in number of air cells within the extrudate and the increase in number of pores (tiny air cells) in the gelatinized starch matrices of the air cell walls with an increase in concentrations of NaHCO₃ (Figs. 1 and 4) may be attributed to an increase in CO₂ released during extrusion processing. The increases in diameters and expansion ratios of the extrudates with an increase in NaHCO₃ concentrations (Table 1) additionally explain the increase in CO₂ released during extrusion processing. Therefore, expansion characteristics of black bean extrudates corroborate SEM observations.
Diameter measurements taken at the node and at the area between the nodes demonstrated that the diameter of these two regions increased proportionally with increased NaHCO₃ concentration in the extrudates, and that there is a significant difference between the control extrudate and extrudates containing NaHCO₃ (Table 1). Also, the standard deviations of the control extrudate diameters and the diameters of the extrudates containing NaHCO₃ were large. However, the standard deviations around the mean diameter within the extrudates containing NaHCO₃ were small within the range of 0.28–0.35 at the node, and from 0.26 to 0.36 in the area between the nodes.

The increase in extrudate diameters is reflected in the expansion ratios. Expansion ratio is a measure of the cross-sectional area expansion of the extrudate. Expansion ratio increased 1.6-fold at the node and 1.4-fold in the area between the nodes for the control extrudate and the extrudate containing 0.1% NaHCO₃, respectively. The difference among expansion ratios increased to 2.0 and 1.8-fold for extrudates containing 0.5% NaHCO₃ at the designated cross-sectional areas. Differences in expansion distribution patterns of extrudates are important to consider in the fabrication of selected snack-type products.

Bulk density, expressed as g/cm³, is also a measurement of expansion of the extrudate. Edwards et al. (1994) indicated that bulk density considers sample weight and the three dimensional changes of the extrudate resulting from expansion at the die. Bulk density is inversely related to expansion ratio, and bulk density decreased with an increase in NaHCO₃ addition from 0.35 to 0.24 g/cm³ for control extrudate and extrudate with 0.5% NaHCO₃, respectively (Table 1). This suggests that the mass of the extrudate became lighter and expanded more with the addition of greater concentrations of NaHCO₃. Bulk densities were similar to those reported for small white bean extrudates.

### Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Diameter (mm) at the nod</th>
<th>Diameter (mm) between the nod</th>
<th>Expansion ratio at the nod</th>
<th>Expansion ratio between the nod</th>
<th>Bulk density (g/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.18 ± 0.13</td>
<td>4.77 ± 0.12</td>
<td>6.70 ± 0.33</td>
<td>5.68 ± 0.28</td>
<td>0.35 ± 0.00</td>
</tr>
<tr>
<td>0.1% NaHCO₃</td>
<td>6.64 ± 0.32</td>
<td>5.71 ± 0.27</td>
<td>11.03 ± 1.06</td>
<td>8.18 ± 0.78</td>
<td>0.32 ± 0.01</td>
</tr>
<tr>
<td>0.2% NaHCO₃</td>
<td>6.88 ± 0.31</td>
<td>5.61 ± 0.36</td>
<td>11.86 ± 1.08</td>
<td>7.90 ± 1.01</td>
<td>0.33 ± 0.00</td>
</tr>
<tr>
<td>0.3% NaHCO₃</td>
<td>6.90 ± 0.35</td>
<td>5.76 ± 0.27</td>
<td>11.92 ± 1.19</td>
<td>8.30 ± 0.79</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>0.4% NaHCO₃</td>
<td>7.25 ± 0.30</td>
<td>6.03 ± 0.29</td>
<td>13.14 ± 1.09</td>
<td>9.10 ± 0.88</td>
<td>0.26 ± 0.00</td>
</tr>
<tr>
<td>0.5% NaHCO₃</td>
<td>7.34 ± 0.28</td>
<td>6.29 ± 0.26</td>
<td>13.48 ± 1.02</td>
<td>9.92 ± 0.82</td>
<td>0.24 ± 0.01</td>
</tr>
</tbody>
</table>
processed with selected moisture contents, barrel temperatures and screw configurations in the range of 0.34–1.11 g/cm³ (Edwards et al. 1994).

**Color Evaluation**

The colorimetric data for parameters of lightness (L*), hue angle (h°_ab) and chroma (C* _ab_) are presented in Fig. 6. Lightness (L*) data were well defined by a linear regression model across concentrations of NaHCO₃ for both extruded and nonextruded black beans. For the nonextruded bean flours, a small difference of 0.5 L* units between the control (0% NaHCO₃ addition) and bean flours with 0.5% NaHCO₃ addition was observed. However, this change was not statistically significant (P = 0.11), as demonstrated by comparing the computed slope to a slope of zero. After extrusion processing, a difference of more than 3 L* units existed between the control bean flours and bean flour extrudates containing 0.5% NaHCO₃. The importance of this change was confirmed by a highly significant (P < 0.0001) and negative slope. As a consequence of the stated differences, the slope of L* across concentrations of NaHCO₃ differed significantly (P = 0.002) for the nonextruded and extruded bean flours as tested with interaction in the model. Additionally, a large decrease of 14 L* units occurred between the control nonextruded and extruded bean flours, which increased to 17 L* units between the control and black bean flours containing 0.5% NaHCO₃.

Three important conclusions (Fig. 6) are confirmed by the statistical tests. First, the addition of NaHCO₃ in the selected concentrations did not significantly affect the lightness of nonextruded bean flours as exhibited by the lack of significance (P = 0.11) of the computed slope. Second, the black bean flours became darker after extrusion processing as exhibited by a large (14 L* units) and highly significant (P < 0.0001) decrease in lightness between the control nonextruded and extruded bean flours. Third, extruded bean flours darkened more with increasing concentrations of NaHCO₃ as exhibited by the negative and highly significant (P < 0.0001) slope. The L* values obtained for extruded bean flours were higher (lighter) than the L* values reported for cereal snack products reported in the range of 45–55 L* value (Wolk 1999). Cereal snack products generally contain reducing sugars that promote nonenzymatic browning, the Millard reaction, which promotes darkening of the cereal snack products.

Hue values (type of color) led to conclusions similar to those observed for L* values. The slope of hue relative to NaHCO₃ was not significantly different from zero (P > 0.5) for the nonextruded flours. Also, this slope was highly significant (P < 0.0001) for the extruded flours, but the slope was positive for hue, indicating an increase in hue angle values (increasing yellow) with addition of greater concentrations of NaHCO₃. As with L*, a
FIG. 6. COLORIMETRIC PARAMETERS FOR LIGHTNESS (L*).

Hue [h^∞_ab = \arctan (b^*/a^*)] and Chroma [C^*ab = (a^2 + b^2)^{1/2}].
large decrease of approximately $17^\circ$ occurred between the control nonextruded and extruded bean flours. The differences decreased to about $13^\circ$ with the addition of the greatest concentration of NaHCO$_3$, indicating that while extruded black bean flours are more reddish-brownish than nonextruded black bean flours, the hue becomes closer to the hue of nonextruded flours after the addition of NaHCO$_3$. Berrios et al. (2002) reported that the pH of extruded bean flours increases with an increase in NaHCO$_3$, and the differences in pH variability were larger than pH variability of nonextruded flours. They attributed the larger increase in pH values of the extruded flours to more effective alkaline reactions of NaHCO$_3$ resulting from the combined effect of multiple extrusion processing operations such as mixing, kneading, shearing, cutting and cooking. Therefore, we conclude that the darker color of the extruded black bean flours was a result of nonenzymatic browning influenced by the pH and heating conditions of the bean flours during extrusion.

Chroma values (vividness of color) for nonextruded flours, as with L* and hue, were not influenced by concentrations of NaHCO$_3$ in the selected range as exhibited by a nonsignificant slope (Fig. 6). For extruded black bean flours, values of chroma did change with the concentration of NaHCO$_3$; however, a cubic polynomial was necessary to describe the trend as opposed to the linear regression which adequately explained changes in L* and hue values. Chroma values for extruded bean flour remained small, about 7 units through 0.1% addition of NaHCO$_3$, increased to a maximum of about 9 units in the range of 0.3 to 0.5% of added NaHCO$_3$ where chroma values leveled off, or started to decline at 0.5% concentration of NaHCO$_3$. The controls containing no added NaHCO$_3$ exhibited nearly a twofold increase in chroma between the nonextruded and extruded bean flours, which increased to approximately a 2.5 difference at the highest concentration of added NaHCO$_3$.

Color determination using L* values and hue angles supports previous visual evaluation of flour extrudates that led to the conclusion that addition of NaHCO$_3$ at concentrations greater than 0.4% were detrimental for extruded bean flours and considered unacceptable for the production of snack-type products (Berrios et al. 2002). It is more difficult to interpret chroma information because the data are not linear. Therefore, chroma may be of less practical use than L* and hue for the evaluation of extruded bean flours.

Because of the lack of extruded legume snacks on the market, there are neither discrete references nor established threshold or cut-off values for color development of an acceptable legume snacks. Therefore, the color data in this study serve as primary information in this regard.
References


