



Effect of the Extrusion on Functional Properties and Mineral Dialyzability from *Phaseolus Vulgaris* Bean Flour

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Abstract. The effects of extrusion conditions on cooking degree, flour dispersion viscosity and mineral potential availability of extruded bean flour were studied. *Phaseolus vulgaris* beans of the agronomic cultivar “Flor de mayo” were ground and dehulled to obtain grits and then extruded at different temperatures (140, 160 and 180 °C) and moisture contents (17, 20 and 23%), according to a bifactorial experimental design. Degree of cooking was estimated by water solubility (WS) and specific mechanical energy (SME). The effect of variables on WS and SME were analysed by surface response methodology. Flour dispersion viscosity and mineral availability (estimated by *in vitro* dialyzability), were also evaluated on selected samples. Results showed that, within the ranges of the variables used for this study, only the effect of temperature was significant on the degree of cooking. No direct correlation was observed between water solubility and SME, although a maximum value of WS corresponded to a range of SME values of 400–500 J/g was observed. Dispersion viscosity decreases as WS increases, so if high calorie density is desired, for instance in order to produce a cream soup formula, bean grits should be extruded at high temperature and as low moisture as possible, in our case 180 °C and 17% moisture. On the other hand, the effects of extrusion variables on iron and zinc dialyzability were not much affected.

Key words: Bean flour, Dialyzability, Extrusion, Functional properties, Mineral availability

Introduction

It is well known that legumes are good protein source, not only because their amino acid composition but also because they can be grown in semiarid regions. Their values become more significant for population with low-income level because its consumption can help to reduce protein calorie malnutrition [1]. Legumes are also a good mineral source [2]. For many people, legumes are staple and are consumed as whole cooked grain or as porridge. Some commercial products based on legume flour, such as powder mix to prepare instant cream soup, are in the market. These kinds of products are formulated adding modified starches to legume flour in order to get a creamy texture.

Extrusion is now considered an appropriate technology to produce a variety of products based on starchy materials. Cereals flours and grits are the main raw material for the production of expanded snacks and precooked flours [3, 4]. The effect of extrusion variables on the properties of extruded cereals has been studied extensively [5–9]. Extrusion cooking has been used to obtain precooked flours

from cereals, legume grains and also from their mixtures, but there is scarce information about the effects of extrusion conditions on functionality and on mineral bioavailability of precooked legume flour.

The mineral bioavailability is defined as the proportion of the total dietary intake that is utilized for normal body function. The major determinant of Fe and Zn bioavailability is the proportion of the nutrient that is absorbed from the gastrointestinal tract. This is greatly influenced by physicochemical and dietary factors in the lumen. The dialyzability method applied to determine Fe and Zn potential availability in this work takes into account these factors and controls final pH during intestinal digestion [10].

In the present work the effects of extrusion conditions on cooking degree, flour dispersion viscosity and mineral dialyzability from an extruded bean flour to be used in cream soup formulas, are studied.

Material and Methods

Bean Grits Preparation

Phaseolus vulgaris beans of the agronomic cultivar “*flor de mayo*” (provided by the Instituto Politécnico Nacional, Durango, México), having a moisture content of 9.61%, were broken in pieces between 3 and 1 mm using a roll mill (Vario-MIAG, Germany), dehulled in an air separator and ground to obtain grits with a particle size between 0.420 and 1.19 mm size. Milling procedure was done according to a mill diagram developed in our laboratory, which permit to obtain grits with minimum production of fine fraction [11]. Grits composition in dry basis, was the following: ash 4.1%, crude protein 25.4%, ether extract 2.5%, starch: 32%, crude fibre 4.56%, iron 46.5 mg/kg and zinc 30.2 mg/kg.

Extrusion Experiments

Grits moisture was adjusted by adding water and mixing in a Brabender (Germany) planetary mixer and the conditioned samples were kept in plastic bags for 1–2 h before extrusion.

The extrusion process was carried out with a Brabender (Germany) 10 DN single screw extruder, using a 3:1 screw compression ratio, a 3-mm die, and a screw speed of 150 rpm.

Extruder barrel temperature and grit moisture were varied according to a two factor experimental design 3^2 , with a triplicate central point, to analyze their effects on the properties selected as responses. Temperature (T) and moisture (M) ranges were 140–180 °C and 17–23% respectively. Extruded samples were left in ambient air until equilibrium (final moisture was approximately 11%). After that, samples were milled in a CICLOTEC mill (UD Corporation, Boulder-Colorado, USA) and kept for the analysis of each response.

Measurement of Responses

a) Extrusion Process. The extruder was operated at full feed in all runs. Torque was obtained in Brabender units (BU). Mass output (Qa), in g/min (feed moisture basis), was calculated by weighing the amount of sample coming out in one minute and determining the moisture content after it was stabilized at room temperature. All samples and data were taken after the stationary state of the extrusion run was achieved, that is, after reaching constant values of Qa and torque.

Specific mechanical energy consumption (SME), in joule/g, was calculated according to González et al. [9], by using the following formula: $SME = 61.3 \times 10^{-3} \times \text{torque (BU)} \times \text{rpm/Qa (g/min)}$.

b) Protein and Solid Water Solubility. Since SME and Water solids solubility (WS) are directly related to cooking degree of extruded starchy materials [12], they were selected as estimators of the state of granule structure (crystallinity and integrity). Considering that after extrusion, water solubility of protein decreases but that of starch component increases, both, solubility of total solids and of non-protein solids, was measured. This was done by dispersing 5 g of flour in 100 ml water, agitating during 30 min and centrifuging at 2000 g; total soluble solids (TSS%) were obtained from supernatant after evaporation in an air oven at 105 °C. Soluble protein, determined by Kjeldhal [13] was also measured in the supernatant. Solubility of total solids was calculated as g soluble solids/100 g of flour (d.b.) and non protein soluble solids (NPSS%) as: $100 \times (\text{g soluble solids} - \text{g soluble protein})/100 \text{ g flour (d.b.)}$

The reported values of: TSS and NPSS are averages of triplicate determinations.

c) Flour Dispersion Viscosity. Three solid concentrations were selected to prepare the extruded bean flour dispersions: 10, 12 and 14%. Their corresponding rheograms at 60 °C were obtained, using a Haake Rotovisco viscometer.

For comparison purposes, a commercial pea cream soup sample was also included, but the levels of solid concentration were 5.9, 7.6 and 10.4%, being 5.9%, the suggested concentration (product label) to be used by consumer. In all cases power law parameters k and n ($\tau = k G^n$), were estimated by regression, from an average rheogram obtained from average τ values, calculated from duplicated rheograms.

d) Observation of Starch Granules. In order to have an idea of the state of starch granules after extrusion, a 10% flour dispersion of extruded samples was observed under normal and polarized light (200x), using a Leitz microscope HM-Pol (Weztlar -Germany).

e) Determination of Mineral Dialyzability (DFe%, DZn%). A modification of the widespread *in vitro* Miller et al. method [14] according to Wolfgor et al. [15] was followed. The samples were prepared to 3% protein concentration (W/W) using distilled water at 70 °C. Aliquots (25 g) of homogenized samples were adjusted to pH 2.0 with 6 N HCl and after addition of 0.8 mL pepsin digestion mixture (16% pepsin (Sigma P-7000) solution in 0.1 N HCl), were incubated at 37 °C during 2 h in a shaking water bath. At the end of pepsin digestion, dialysis bags containing 20 mL 0.19 M PIPES (piperazine-N,N'-bis[2-ethanesulfonic acid] disodium salt) buffer (Sigma P-3768) were placed in each flask and were incubated for 50 min in a shaking water bath at 37 °C. Pancreatin-bile mixture (6.25 mL of 2.5% bile (Sigma B-8631), 0.4% pancreatin (Sigma P-1750) solution in 0.1N NaHCO₃) was then added to each flask and the incubation continued for another 2 h. Then, bag contents were weighed and analyzed for its mineral content by flame atomic absorption spectroscopy (AAS). Assessment of minerals in samples was made by AAS after dry ashing [16]. Mineral dialyzability was calculated from the amount of each dialyzed mineral expressed as a percentage of the total amount present in each sample.

Dialyzable Mineral (%) = [D/(W x A)] × 100; where: D is the total amount of dialyzed mineral (μg); W is the weight of sample (g) and A is the concentration of each mineral in the sample ($\mu\text{g/g}$).

Statistical Analysis

Surface response methodology [17] was used to determine the effects of extrusion variables (T and M) on SME and water solids solubility (total and non proteins solids). Analysis of variance (ANOVA) and LSD test (Least Significant Difference) were also used to determine the statistical significance of differences among some selected samples regarding the flour suspension viscosity and mineral dialyzability (Statgraphics plus 3.0).

Table 1. Water solubility: non protein soluble solids (NPSS%), total soluble solids (TSS%) and specific mechanical energy (SME) values corresponding to each extrusion condition

Temperature (°C)	Moisture (%)	NPSS (%)	TSS (%)	SME (J. g ⁻¹)
140	17	28,3	25,1	280
140	20	31	26,1	350
140	23	33,6	29	380
160	17	43,5	38	700
160	20	39,5	33,4	530
160	20	41,4	35,2	600
160	20	40,82	34,6	580
160	23	38,5	33,5	380
180	17	54	45	480
180	20	50	43	420
180	23	47	40,5	400

Results and Discussion

Effects of Extrusion Variables on Degree of Cooking

Table 1, shows the results obtained for TSS%, NPSS% and SME, corresponding to each extrusion condition and Table 2 shows the corresponding ANOVA for those results. It is observed that only the polynomial terms T and T × M, are significant for both types of solubility (TSS% and NPSS%), being T the most significant one for both responses ($p < 0.0017$ and $p < 0.0022$, respectively). For SME the only significant term is T² ($p < 0.0175$), although p value corresponding to M is no too low ($p < 0.0768$). This means that in the range of variables used in this study, the effects of T are more important than those of M. However, it has been shown that M is the most important variable when maize grits alone are extruded [9]. An explanation for this difference is that, being bean cotyledon structure softer than that of maize endosperm, friction level generated by

bean grits inside the extruder would be lower than that caused by maize grits. Moreover, the extruded sample observation under polarized light showed that those extruded at 140 °C contained an important proportion of birrefringent starch granules. On the other hand, none birrefringent granules were observed in those samples extruded at T 160 and 180 °C. These results indicate, that at 140 °C, the fluid flow mechanism is not fully established and consequently part of the grits particles reaches the die without the starch granule disruption had been produced. It is well known that the granule disruption process is the basis for extrusion cooking of starchy materials. In fact, the lowest torque values (not shown) were obtained for 140 °C extrusion runs and so the lowest SME values corresponded to these samples. This effect of particle hardness on the degree of cooking of starch has been observed by Robutti et al. [18], with maize grits obtained from cultivars of different endosperm hardness. They explained that harder maize cultivars would cook and become fluid more rapidly than softer ones. In a work related to maize grits extrusion operation, González et al. [19] found, that for some combination of die diameter, screw compression ratio and grit moisture (for example: a die of 4mm, a grit moisture of 14% and a screw compression ratio of 3:1), the fluid flow transport mechanism could not completely be achieved and in such cases a significant reduction in degree of cooking (water solubility) was observed.

Figures 1 and 2 show the response surfaces, for TSS% and SME respectively. It is observed the clear effect of temperature on TSS%, which increases continuously as T increases, confirming results obtained by other authors [5, 20, 21, 22].

On the other hand, the effect of T and M, on SME seems more complex. The lowest SME values corresponded to those samples extruded at 140 °C (which also showed the lowest torque values), then as T increases, fluid flow

Table 2. ANOVA applied to water solubility: non protein soluble solids (NPSS%), total soluble solids (TSS%) and specific mechanical energy (SME) values corresponding to the experimental design for extruded bean samples

Source of variation	Degree of freedom	Sum of squares					
		NPSS	TSS	SME			
T	1	562.602	*	388.815	*	14,016.7	
M	1	7.4817		4.335		15,000.0	
TxM	1	37.8225	**	17.64	**	8,100.0	
T ²	1	0.078167		0.35875		72,534.9	**
M ²	1	0.26542		1.71875		202.807	
Lack of fit	3	5.86908		6.99158		37,443.9	
Pure error	2	1.88667		1.68		2,600.0	
Total	10	615.949		421.247		149,218.0	
R ² (%)	–	98.7408		97.9415		74.8862	

Note. T: Extrusion Temperature; M: Grit Moisture.

* $P < 0.01$.

** $P < 0.05$.

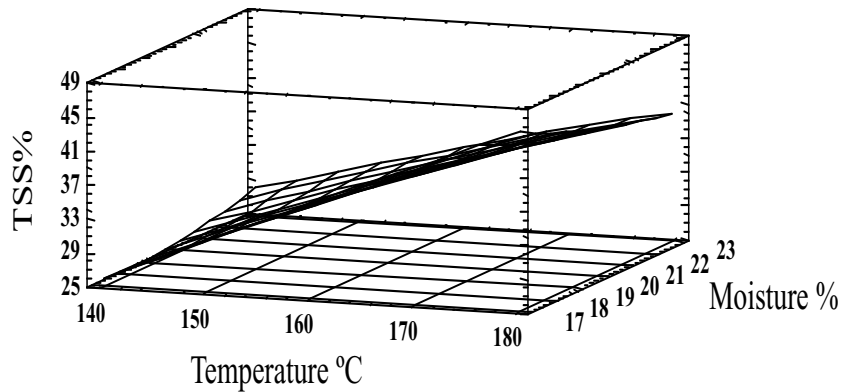


Figure 1. Surface Response for total soluble solids (TSS%).

conversion can be completed and higher torque levels are obtained; so SME increases as T increases. But, as T increases melt viscosity decreases, then at some point SME starts to decrease. Again the incomplete solid flow transformation is the reason why the expected direct correlation between water solubility and SME (as normally is found when maize grits is extruded) is not obtained. Figure 3 shows the existence a maximum value of WS (TSS%) corresponded to a range of SME values of 400–500 J/g.

Then, for this particular extrusion variable combination used in our experiments, friction level generated by bean grits having moisture content $\geq 17\%$ would be insufficient to promote fluid flow conversion in the bean grit mass at 140 °C. The most important portion of the energy used for cooking comes from the heater across the barrel surface. At 160 °C or higher and at these moisture levels, the transformation of solid transport mechanism in the fluid flow one is promoted allowing the development of the extrusion cooking process [19]. Because of these, to reach an adequate cooking degree of extruded bean, a higher amount of energy from outside is needed in comparison to maize grits.

Dispersion Viscosity

When flour dispersions were done with each of the extruded samples, those extruded at 140 °C, showed a clear

instability. Insoluble particles with poor hydration capacity settle very fast impairing the dispersion appearance and smoothness in the mouth. These samples are considered not acceptable to be used for cream soup formulations. Four samples were selected for viscosity measurements: 160/17, 160/20, 180/17 and 180/20 (°C/%).

Table 3 shows power laws coefficients (K and n) and viscosity at 100 s^{-1} of dispersions corresponding to each solid concentration of a commercial sample and of the extruded bean sample, excluded those obtained at 140 °C. Results show that power law regression model fitted very well all the rheograms obtained for the three solid concentrations assayed. According to Shama and Sherman [23], a shear rate of 100 s^{-1} was selected to compare sample dispersions viscosity, since this gradient velocity is in the range of that in the mouth when a fluid food, such as a cream soup would be evaluated.

In order to compare more clearly the effect of solid concentration on viscosity, their values are shown in Fig. 4.

When water solubility (TSS% or NPSS%) values are compared with those of viscosity, it is clear that as water solubility increases, dispersion viscosity decreases and that the lowest viscosity value is obtained with sample extruded at 180 °C and 17%. These results are in agreement with those obtained for extruded maize grits [24, 25]. These results allow us to say that if high caloric density is desired

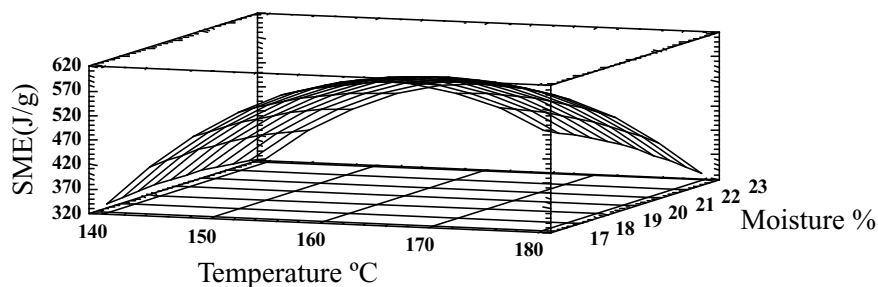


Figure 2. Surface Response for specific mechanical energy consumption (SME).

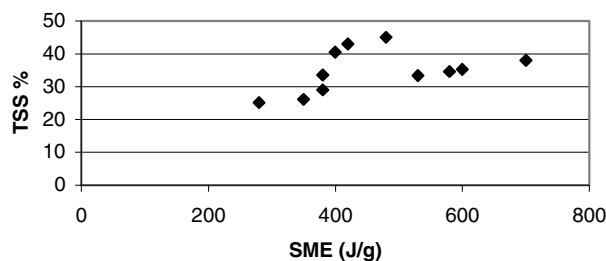


Figure 3. Relationship between specific mechanical energy (SME) and total soluble solids (TSS%) corresponding to the extrusion condition used: Brabender 10 DN single screw extruder, 3:1 screw compression ratio, 3-mm die, 150 rpm screw speed, temperature (140, 160 and 180 °C), moisture (17, 20 and 23%).

for a cream soup formula, bean grits should be extruded at high temperature and as low moisture as possible; in our case 180 °C and 17% moisture; on the contrary the lowest caloric density is obtained at 160 °C and higher moisture levels.

Mineral Dialyzability

Table 4 shows iron and zinc dialyzability (FeD% and ZnD%, respectively) from bean grits and extruded samples at extrusion conditions corresponding to the highest and lowest T and M. In general, extrusion conditions did not affect FeD%. Only the extruded sample with the lowest cooking degree resulted significantly different from that having the highest one. Fairweather-Tait et al. [26] and Kivistö et al. [27] studied the effect of extrusion on mineral bioavailability in humans and observed that extrusion did

Table 3. Power law model coefficients (K and n) and viscosities (at 100 s⁻¹) of dispersions, corresponding to each solid concentration of extruded and commercial samples. T: temperature; %M: moisture; %S: solids

Sample T °C/%M/%S	K	n	$\eta_{100\text{ s}^{-1}}$ [cp]
180/20 10%	0,316	0,75	0,97 ^b
180/20 12%	2.01	0.53	240 ^f
180/20 14%	4.07	0.51	430 ^j
160/20 10%	0.76	0.64	140 ^d
160/20 12%	2.12	0.61	350 ⁱ
160/20 14%	5.39	0.53	650 ^l
180/17 10%	0.25	0.75	75 ^a
180/17 12%	1.85	0.51	190 ^e
180/17 14%	3.22	0.50	335 ^h
160/17 10%	0.41	0.76	130 ^c
160/17 12%	1.79	0.62	310 ^g
160/17 14%	6.51	0.47	570 ^k
C 5.9%	0.97	0.441	74 ^a
C 7.6%	2.761	0.361	139 ^d
C 10.4%	7,58	0.329	341 ^{hi}

Note. Values with different letter(s) within columns are significantly different at $p < 0.05$.

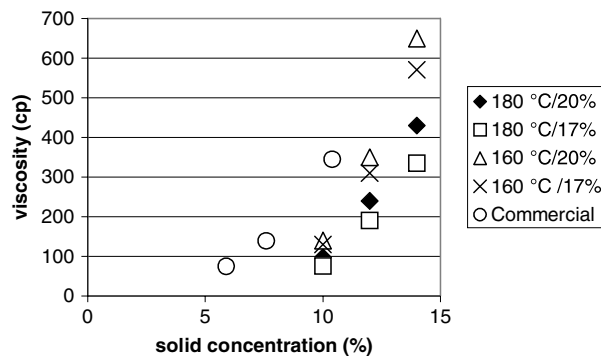


Figure 4. Dispersion Viscosities at 100 s⁻¹, corresponding to each solid concentration of extruded and commercial pea soup samples.

not affect iron bioavailability, but the matrixes studied were different from that in our work. Legumes have phytates and polyphenols, which inhibit iron and zinc absorption [28]. The extrusion process could degrade phytates, but the final level depends on the extrusion conditions [29, 30]. Moreover Ummadi et al. [29] also observed an increase in FeD% from legumes, depending on extrusion conditions.

Regarding zinc, the extrusion slightly decreases ZnD%, depending on the extrusion conditions. Drago et al. [31] also observed that extrusion decreases ZnD% from soy grits. However this effect on ZnD% is small and the potential availability is considerably high.

Conclusions

The effect of extrusion temperature on the degree of cooking becomes more important than bean grits moisture content. To get extruded bean samples having a high degree of cooking, needed to formulate a cream soup base powder, it is very important to select extrusion variables in order to avoid such extrusion variable combinations that fluid flow transport mechanism can not be completely achieved. By using extrusion conditions such as 180 °C and 17% moisture, much lower dispersion viscosity is obtained in comparison with that of commercial pea cream soup. So if high calorie

Table 4. Iron and zinc dialyzability (D%) at different extrusion conditions

Temperature (°C)	Moisture (%)	DFe%	DZn%
140	17	10.2 ± 0.3 ^{a,b}	35.2 ± 3.1 ^{a,b}
140	23	8.6 ± 0.3 ^a	32.8 ± 0.3 ^a
180	17	10.7 ± 1.6 ^b	33.3 ± 2.7 ^a
180	23	10.1 ± 0.3 ^{a,b}	35.7 ± 0.8 ^{a,b}
Raw grits		9.6 ± 1.1 ^{a,b}	37.9 ± 1.8 ^b

Note. Values with different letter(s) within columns are significantly different at $p < 0.05$.

x ± SD (n = 4).

density is desired, for instance in order to produce a cream soup formula, bean grits should be extruded at high temperature and as low moisture as possible. Mineral availability was lightly affected for extrusion at the extrusion condition used in this study, but these small effects could not be significant *in vivo*.

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