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Effect of pressure cooking on the antioxidant activity of extracts from three common bean (*Phaseolus vulgaris* L.) cultivars

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Abstract

Common bean is a traditional legume that is becoming more attractive because of its dietary and associated health benefits. Flor de Mayo M38, Pinto Villa and Bayo Victoria, three common bean cultivars developed in Mexico, were tested for phenol content and free radical scavenging activity before and after autoclaving. Independent analysis of seed coat and cotyledon was performed for each cultivar. Longer cooking times enhanced diffusion of phenols from seed coats to cooking water and from there to cotyledons. Cooking waters showed a remarkable activity similar to crude seed coats extracts although their phenol content was 80% lower.

Keywords: Antioxidant activity; Common beans; Cooking; Polyphenols

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is a traditional food in human diet, low in fat and rich in proteins, vitamins, complex carbohydrates and minerals. Consumption of dry beans has been linked to reduced risk of diabetes, obesity (Geil & Anderson, 1994), heart disease (Anderson et al., 1984) and colon cancer.

Beans as legumes are a good source of soluble dietary fiber. This prebiotic ingredient composed mainly of oligosaccharides slowly releases carbohydrates, thus, regulating gastric emptying and the rate of digestion and absorption. However, their wider use is somehow limited by the presence of antinutritional factors, which might produce adverse effects for human and animal nutrition. Some examples of these compounds are enzyme inhibitors, lectins, phytates, cyanoglycosides and phenolics (Martin-Cabrejas et al., 2004).

Many publications on *P. vulgaris* have focused on antinutritional aspects of seed coat polyphenols such as condensed tannins (Elias, Fernández, & Bressani, 1979). However, it has been reported that polyphenols have anticarcinogenic and antioxidant properties (Gamez et al., 1998). According to Hagerman et al. (1998), condensed and hydrolyzable tannins of relatively high molecular weight have also shown to be effective antioxidants with greater activity than simple phenols. It is generally believed that antioxidants scavenge free radicals and reactive oxygen species and can be extremely important in inhibiting oxidative mechanisms that lead to degenerative diseases (Cardador-Martínez, Loarca-Piña, & Oomah, 2002). Several studies had been conducted with bean extracts in order to know its potential antioxidant activity. For example, in Tsuda, Ohshima, Kawakishi, and Osawa (1994), antioxidant activity was evaluated in pigments isolated from common beans. Recently, antioxidant activity was reported

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(Benninger & Hosfield, 2003) in extracts, condensed tannins and pure flavonoids from colored genotypes of common bean seed coats. Unfortunately, there are few studies about the effect of thermal processing in the nature of phenols and their biological effects. Such is the case of some works (Jiratavan & Liu, 2004; Martin-Cabrejas, Esteban, Perez, Maina, & Waldron, 1997) that relate hard-to-cook phenomena with phenolic content and the antioxidant activity after processing green beans. Although there is currently a remarkable interest in the study of phytochemical profiles and antioxidant activities of legumes, the question on how processing impacts antioxidant activity of dry beans is still unanswered. Therefore, the goal of this work was to evaluate the effect of pressure cooking in the antioxidant activity of extracts from three common bean cultivars.

2. Materials and methods

Common bean cultivars 'Flor de Mayo M38' (Acosta-Gallegos et al., 1995), 'Pinto Villa' (Acosta-Gallegos et al., 1995) and 'Bayo Victoria' were cultivated and harvested at the 'Valle del Guadiana' Experimental Station of Instituto Nacional de Investigaciones Forestales Agricolas y Pecuarias (INIFAP) under irrigated conditions.

The weights of 100 randomly chosen seeds were determined and reported as g/100 seeds. The seed coat for each bean was weighted and registered. The percentage of seed coat was calculated as a function of the total weight of bean and multiplied by 100. The color of beans was described according to visual characteristics.

The general experimental methodology followed in this study is shown in Fig. 1. Seed coats were removed manually with the aid of forceps and a knife. Samples were saved into hermetic plastic bags and stored under dry conditions before use. Bean flour was prepared from separated cotyledon and seed coat for each common bean cultivar. Every sample (30 g) was crushed in a lab mill (Ohaus) and kept dry before use.

Pressure cooking of beans was performed by autoclaving, according to conditions described in Table 1. Cooking time was set following sensorial method proposed by Elias et al. (1979). After the cooking process, beans were let to cool down and separated from cooking water. Then, coat and cotyledons were separated manually as done with crude beans and kept under dark storage. Cooking water was concentrated under vacuum and freeze dried.

Samples were dewaxed with hexane and later extracted with 70% acetone in similar manner. Every sample (15 g) plus 300 mL of solvent was shaken in Erlenmeyer flasks for 24 h in a Gallenkamp stirrer at 50 rpm. Later they were filtered and crude extracts concentrated in rotary evaporator at reduced pressure and low temperature (40 °C). Concentrated extracts and cooking waters were freeze dried in an Edwards lyophilizer (8 mbar, −50 °C, 48–72 h).

Total phenols were determinated by the Folin–Ciocalteu method using a Cary Varian UV–Vis spectrophotometer. The antioxidant activity was evaluated by the DPPH

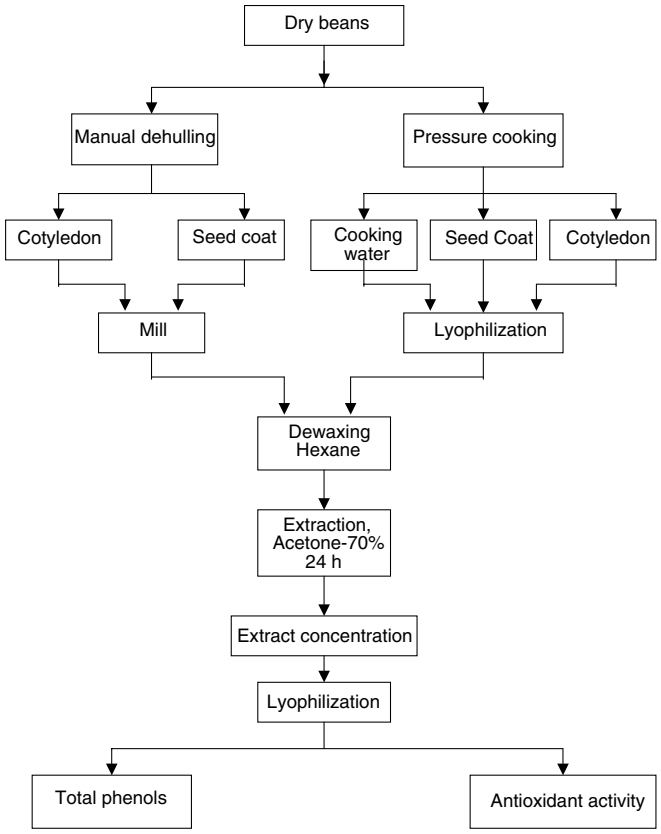

Fig. 1. Flow chart for experimental methodology.

Table 1
Pressure cooking conditions for autoclaving common beans

Cultivars	Temperature (°C)	Pressure (kPa)	Time (min)
Pinto Villa	121	103.421	7
Bayo Victoria	121	103.421	60
Flor de Mayo M38	121	103.421	5

(2,2-diphenyl-1-picrylhydracyl) method, which measures the relative capacity of extracts in scavenging free radicals (Brand-Williams, Cuvelier, & Berset, 1995). Solutions from each extract at different concentrations (5000, 500, 50 and 5 mg/L) were prepared for DPPH assay.

Experiments were performed following a factorial design and statistical results were analyzed with ANOVA and Tukey tests ($p < 0.05$).

3. Results and discussion

Physical characteristics of beans are shown in Table 2. The color of beans used in this experiment was clear (beige for Bayo to Cream with brown mottles for Pinto Villa). Two race were used (Race Jalisco and Durango). The weight of the beans used indicates two types of beans (medium and small); this classification is according to that reported by De Mejía et al. (2003) for the same cultivars.

Phenolic content in tested beans is shown in Fig. 2. Most studies about common beans have been conducted in the

Table 2
Physical characteristics of the common beans used in the experiment

Cultivar	Seed color	Seed weight (g/100 seed)	Seed coat content (%)	Race
Flor de Mayo M38	Cream with pink mottles*	30 ± 1*	8.81 ± 0.18*	Jalisco*
Pinto Villa	Beige*	58 ± 1*	8.29 ± 0.18	Durango
Bayo Victoria	Cream with brown mottles*	40 ± 2.5*	7.98 ± 0.14	Durango

* Statistical differences ($p \leqslant 0.05$).

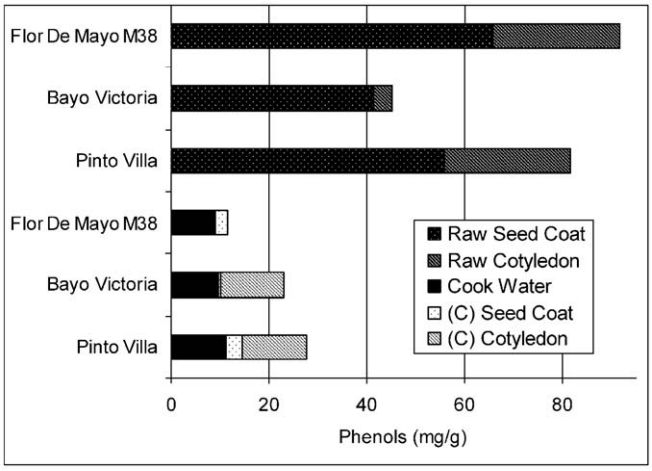

Fig. 2. Phenols content in crude and cooked beans calculated as equivalent mg of catechin/g of bean.

seed coat, where polyphenols are concentrated. For this study, cotyledons were separated and analyzed too. In De Mejía et al. (2003), same cultivars were analyzed (Pinto Villa, Bayo Victoria and Flor de Mayo M38) but they were not cooked. In the case of Flor de Mayo M38, the value reported by De Mejía et al. (2003) was 35.5–44.2 mg of Catechin/g sample. This value is lower than the one obtained in present study, although the difference could be explained in function of weather conditions and geographical location (Ma & Bliss, 1978). In the other two varieties (Bayo Victoria y Pinto Villa), values reported by De Mejía et al. (2003) were also lower. However, Barampama and Simard (1993) and Reddy, Pierson, Sathe, and Salunkhe (1985) reported similar values to those obtained here. In general, when evaluation of phenols is made by separating seed coat and cotyledon, the values obtained are higher than those from the complete seed (Guzmán-Maldonado, Castellanos, & de Mejía, 1996).

Significance of phenolic content maybe discussed from two points of view. First, evaluating the negative effects of consumed phenols and second, estimating their positive contribution to health. In this sense, it is evident that Flor de Mayo M38 shows higher phenols concentration in seed coat than the other two cultivars, but in cotyledon phenolic content is relatively high in Flor de Mayo M38 and Pinto Villa too. These results are different to those reported by Deshpnade (1992), who affirm that phenols are found mainly in seed coats and their concentration in cotyledons is meaningless.

In general, it has been said that common beans having light color show lower content of phenols in comparison to dark beans (Barampama & Simard, 1993). However, in the present study Flor de Mayo M38 having relatively low pigmentation (see Table 2) shows the highest value in phenolics. Relationships between color and phenols are controversial. While in Barampama and Simard (1993) the authors found a relationship between color and phenol content, Guzmán-Maldonado et al. (1996) did not find any. There are reports (De Mejía et al., 2003) with more relationships between cultivar lines and phenolic content. For example, it was reported that Jalisco race shows higher levels of phenols than Durango race. This relationship was clearer than the relationship between color and phenols. In this report, we confirmed that Flor de Mayo M38, a Jalisco race cultivar, has higher content of phenols than Bayo Victoria, a Durango race cultivar. Another point of view was explained by De Mejía et al. (2003). They said that most of the phenols are located in the seed coat and smaller seeds usually have more seed coat area by weight than larger seeds thus, smaller seeds could have a higher phenol concentration, which was confirmed in this experiment (See Table 2 and Fig. 2). However, phenol content variation may still be explained as a function of several local variables as growing site and cultivation practices. It is important to highlight the significative phenol content in the three common beans cultivars.

Realistically, common beans are consumed cooked, not crude. Thus, it is important to know what happen, to polyphenols during pressure cooking. The results obtained with regard to phenols content are shown in Fig. 2. Phenolic content in common beans under pressure cooking, was reduced drastically by 90% (seed coat) or more. This result agrees well with the reports of Barroga, Laurena, and Mendoza (1985) who found that boiling and cooking reduced the in the amount of phenols in legumes by 73%. However, Fernández, Elias, Braham, and Bressani (1982) found an increase in the level of phenols in cooked common beans. In the present experiment, Bayo Victoria shows higher value in cooked cotyledon compared to crude ones. According to Barroga et al. (1985), a minor amount of phenols can be explained by a lixiviation phenomenon that drives phenols into the cooking water. This process is a function of temperature (i.e., at higher temperature, higher lixiviation rates), and will promote diffusion of phenols into cotyledons too. This phenomenon can occur in Bayo Victoria beans, which undergo a longer cooking time (60 min) than the other two cultivars. Similar results were reported by Vidal-Valverde et al. (1994) for cooked lentils.

The evaluation of antioxidant effect by DPPH* method is shown in Fig. 3. No statistical differences were found in

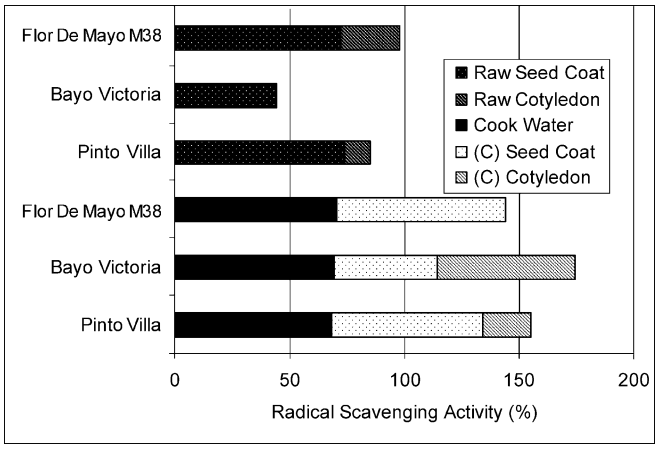

Fig. 3. Antioxidant activity of common bean extracts evaluated by the DPPH* method. Values are presented as % of radical scavenging activity (% RSA) evaluated at 5000 μg/g of sample at 60 min.

crude cotyledons activities. Also crude seed coats did not show statistical differences. It is interesting to note that concentration does not have relative influence, because low levels of extract (5 mg/L) showed similar effect in free radical trapping than higher levels of extract (5000 mg/L).

Although crude common beans showed no differences in their antioxidant activity, after cooking they did it. Thus, the radical scavenging activity (%RSA) of seed coats was related with cooking time. At higher cooking time, minor capacity of seed coat extracts was observed for trapping free radicals. This is caused in part by diffusion of phenols to cooking water and cotyledon and the formation of complex phenol–protein, which, is not an active component to produce antioxidant effects. The %RSA of crude cotyledon was around 0–25%, where Flor de Mayo showed the highest activity, Pinto Villa was moderate and Bayo Victoria was the lowest. However, comparing these results with those from seed coats, it is clear that for Bayo Victoria its major activity was found in cotyledon.

At the time of cooking, Flor de Mayo M38 cultivar showed important changes, its cotyledon did not have %RSA, although this bean in crude showed higher %RSA. After cooking their activity was apparently lost. Pinto Villa cotyledon almost doubled its free radical trapping capacity (20% vs 11% in crude) after the cooking process. Now in Bayo Victoria beans the change was notable, the level of %RSA in crude was minimum but after cooking it increased to 65%. This result indicates that at higher cooking time, phenols from seed coat diffuse to cooking water and from there to cotyledons. The first step, the diffusion of phenols to cooking water, diminishes the concentration of seed coat extracts and their activity in Flor de Mayo M38 cultivar (which has lower cooking time), but when cooking progresses, phenols can diffuse into cotyledon. This phenomenon causes outstanding increase of activity in cotyledon from Bayo Victoria bean (60 min of cooking).

Differences in the content of phenols in cooking waters were not significative. It is interesting to note that although the level of phenols was low for cooked beans, their radical scavenging capacity was similar to that measured for crude seed coats and higher than that measured for crude cotyledons.

However, cumulative % radical scavenging activity (seed coat + cotyledon + cooking water) for cooked beans was higher than that for crude beans (see Fig. 3) independently of the fact that in cooked beans the phenol level was lower and independently of the cultivar.

In the case of crude or cooked seed coats and cotyledons, and cooking waters, there is no straight relationship between phenol content and %RSA. More studies are necessary to identify bioactive components in common beans and their nature for trapping free radicals.

4. Conclusions

The results obtained indicate great influence of cooking pressure time in phenols distribution (seed coat, cotyledon and cooking water). The important activity of phenols present in cotyledon extract was found in Bayo Victoria cultivars, while cotyledon extracts from Flor de Mayo M38 cooked for shorter time have not shown any activity. Values of %RSA found in cooking waters were similar to those from crude seed coat extracts, which have the highest antioxidant activity. Cumulative values of %RSA for cooked beans were higher than those of crude beans independently of cultivar.

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