



Original/Otros

Glycemic response to Carob (*Ceratonia siliqua L*) in healthy subjects and with the *in vitro* hydrolysis index

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Abstract

The purpose of this study was to determine the *in vivo* glycemic index of carob tablets with healthy subjects and to determine the *in vitro* glycemic index of carob tablets and carob flour by the hydrolysis index. Seven healthy volunteers consumed portions of carob tablets containing 26g of available carbohydrate. Their capillary blood was taken at intervals after carob or glucose consumption. The glycemic hydrolysis index by an *in vitro* technique was based in the release of glucose after enzymatic treatment of carob tablets and carob flour. The determination of the fiber content was performed using the enzymatic-gravimetric method. By the *in vivo* determination, the estimated glycemic index of carob tablets could be considered low (≤ 55). By the *in vitro* determination, the estimated glycemic index ranged from 40.1+0.02 of carob tablets to 40.6+0.05 of carob flour. The total fiber values obtained for carob flour samples were from 42.6% \pm 0.49 to 42.9% \pm 0.68 with no statistical significant differences between samples. Carob tablets and carob flour could be classified as low glycemic index food and low glycemic load food. Carob flour is a high fiber food, containing mainly high levels of insoluble fiber.

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Key words: Glycemic index. Glycemic load. Carob. Dietary fibers. *Ceratonia siliqua L*.

RESPUESTA GLUCÉMICA DE ALGARROBO (CERATONIA SILIQUA L) EN SUJETOS SANOS Y CON EL ÍNDICE DE HIDRÓLISIS *IN VITRO*

Resumen

El presente estudio tuvo como objetivos determinar el índice glucémico *in vivo* de barras de algarroba en individuos saludables y determinar el índice glucémico *in vitro* de barras de algarroba y harina de algarroba, utilizando el índice de hidrólisis. Siete voluntarios saludables consumieron porciones de barras de algarroba conteniendo 26 g de carbohidratos. Se han recolectado muestras de sangre capilar tras la ingestión de barras de algarroba y de glucosa. El índice de glucosa *in vitro* se basó en la liberación de glucosa tras el tratamiento enzimático de barras de harina de algarroba. La determinación de fibras se realizó por el método enzimático-gravimétrico. Por la determinación *in vitro*, el índice glicémico estimado puede ser considerado bajo (≤ 55). Por la determinación *in vitro*, el índice glicémico varió entre 40.1 \pm 0.02 (barras) y 40.6 \pm 0.05 (harina). El tenor de fibra alimentaria encontrado fue del 42.6% \pm 0.49 a 42.9% \pm 0.68, sin diferencias estadísticas entre las muestras. Las barras de harina de algarroba pueden ser clasificadas como alimentos de bajo índice glicémico y de baja carga glucémica. La harina de algarroba presenta alto tenor de fibras alimentarias, sobre todo fibras insolubles.

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Palabras clave: Índice glicémico. Carga glucémica. Algarrobo. Fibras dietéticas. *Ceratonia siliqua L*.

Abbreviations

GI: Glycemic index.
GL: Glycemic load.
MT: More toasted.
LT: Less toasted.
AUC: Concentration-over-time-curve.
HI: Hydrolysis index.

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Introduction

Carob, also called algarroba, locust bean and St. John's bread, is obtained from the fruit of the carob tree (*Ceratonia siliqua* L.) that is eaten in many Mediterranean countries in culinary preparations of beverages and confectionery. The fruit pod, after removal of the seeds, produces the carob powder or carob flour. Carob powder is a natural sweetener with flavor and appearance similar to chocolate, being used as a chocolate or cocoa substitute. The advantage of using carob as a chocolate substitute resides in that carob is an ingredient free from caffeine and theobromine. The carob seeds contain a white and translucent endosperm, called carob gum or locust bean gum that is utilized due to its gelling properties. Carob is rich in polyphenols and therefore contains biological effects including antioxidant actions (Yousif and Alghzawi, 2000; Dakia et al., 2007; Bengoechea et al., 2008; Hajaji et al., 2011).

High glycemic index (GI) and glycemic load (GL) have been proposed to be associated with increased risk of chronic diseases. High GI food intake may elevate postprandial blood glucose levels, leading to high insulin demand. Some studies have shown that the consumption of low glycemic index food improves blood glucose control, lipid profile and lipoprotein concentrations. Slowly digested carbohydrates are generally considered to be beneficial for the dietary management of metabolic disorders, including diabetes and hyperlipidemia (Lottenberg, 2008; Parada and Aguilera, 2009; Youn et al., 2012). The adoption of GI and GL concepts allows the control of postprandial glycemia through dietary measures. Controlling postprandial glycemia is an important target in maintaining health and preventing diseases. For this reason, there is a growing interest in the promotion of diets that evaluate not only the absolute amount of energy or nutrient to be ingested, but are also focused on the postprandial response (Colombani, 2004).

Dietary fiber has a physiological impact on the gastrointestinal tract, since it has the ability to become viscous, kidnap water, bind to bile salts and minerals, and might be degraded by intestinal flora. Thus, it affects gastric emptying, excretion of bile salts, intestinal transit, absorption of nutrients, growth of intestinal flora and reduction of energy intake. Dietary fibers are a vital part of a healthy diet, as diets rich in fibers could decrease the risk of certain non-transmissible chronic diseases and these benefits are enhanced when this diet is associated with low GI foods (Brownlee, 2011; Latulippe, et al., 2013).

Despite of the known beneficial properties of carob, large quantities of it are still discarded. Some carob confectioneries are produced and it is of great importance to determine their levels of dietary fibers and the impact of these products in the glycemic control.

This study aimed to determine the *in vivo* glycemic index of carob tablets, to determine the *in vitro* glycemic index of carob tablets and carob flour and to deter-

mine the content of soluble and insoluble fiber present in carob flour.

Subjects and methods

In vivo study

Seven healthy volunteers, aged between 18 - 55 years, were recruited for this study. Informed consent was received from all subjects and the Ethics Committee of Federal University of Parana, Brazil, gave approval for the study, according to the National Committee for Ethical Research of the Brazilian Health Ministry (CONEP) guidelines.

Portions of carob tablets (Carob House, Brazil) containing 26 g of available carbohydrate and 26 g of glucose were provided to volunteers (Foster-Powell et al., 2002; Menezes et al., 2009). Capillary blood samples were taken at 0, 15, 30, 45, 60, 90 and 120 min, after fasting of 10-12 hours. During the test, volunteers were allowed to consume water.

In vitro study

The glycemic index of carob flour occurred only *in vitro* due to the difficulty of eating large amounts of this product for the *in vivo* test. The *in vitro* method for evaluating hydrolysis index was determined following Goñi et al. (1997) and Walter et al. (2005).

Samples containing 300 mg of carob flour or carob tablets were dissolved or sliced, grinded and dissolved in 10 ml of distilled water. The pH of the samples was adjusted with sodium hydroxide, after which 100 μ l of protease was added. Then, 0.2 ml of ethanol (85%v/v) in 3ml of phosphate buffer (pH 6.0) was added and 100 μ l of α -amylase enzyme. Samples were incubated at 37° C in a shaking water bath. After, 1 ml aliquot samples were taken from each tube at 0, 15, 30, 45, 60, 90 and 120 min. These aliquots were heated at 100° C for 15 min, and refrigerated in the end time of the incubation. Then, 3 ml of sodium acetate buffer (pH 4.75) were added to each aliquot and 80 μ l of amyloglucosidase were used to hydrolyze the digested starch into glucose after 45 min at 60° C. Triplicated aliquots were incubated with colorimetric enzymatic kit Glucose-PP (ANALISA®) to determine the concentration of glucose in each aliquot.

Dietary fiber analysis

Portions of two types of carob flour (Carob House, Brazil) were studied, they differed by the toasting degree, where one type was more toasted (MT), which is intended for preparation of carob tablets, and the other was less toasted (LT), which is intended for consumption in beverages. The determination of the

fiber content was performed using the enzymatic-gravimetric method, recommended by AOAC (1995) and was carried out with thermostable alpha-amylase (pH 6, 100° C, 30 min), protease (pH 4.5, 60° C, 30 min) and finally amyloglucosidase (pH 4.5, 60° C, 30 min). For determination of total fiber, it was added 250 ml of warm 95% ethanol and left covered to stand for 1 hour at room temperature. The ethanolic solution was filtered under a slight vacuum. The residue was washed with ethanol 78%, ethanol 95% and acetone. Then, it was dried overnight in an oven at 105°C, cooled and weighed. Protein content was determined by the Kjeldahl method. For determination of soluble and insoluble fiber, after the last incubation, the residue was filtered and washed with ethanol and acetone for the analysis of insoluble dietary fiber and with ethanol for soluble dietary fiber.

Data analysis

The area under the glucose curve has been calculated as the incremental area under the blood glucose response curve, ignoring the area beneath the fasting concentration, for the *in vivo* study (Araya et al., 2002).

The area under the curve for carob tablets is expressed as a percent of the mean response to the standard food (glucose), taken by the same subject, repeated three times with each individual, and the resulting values are averaged to obtain the GI value for the food. The values were plotted on a graph and the area under the concentration-over-time-curve (AUC) was calculated geometrically by applying the trapezoid rule methodology, described by FAO/WHO (1998).

The glycemic load is calculated by multiplying the amount of carbohydrate in food portion by the glycemic index. Thus, the glycemic load of a typical serving

of food is the product of the amount of available carbohydrate in that serving and the GI of the food (Foster-Powell et al., 2002).

The rate of carbohydrates digestion for the *in vitro* analysis was expressed as the percentage of carbohydrate hydrolyzed at different times (0, 15, 30, 45, 60, 90 and 120 min.). The areas under hydrolysis curves (AUC, 0 – 120 min.) were calculated. The hydrolysis index (HI) values were calculated as the relation between the AUC of the specific sample compared to the AUC of glucose, as reference food. The glycemic index was determined by the mathematical model proposed by Goñi et al. (1997), following the equation $GI = 39.71 + 0.549HI$.

The Student's "t" test was used and a significance level of $p < 0.05$ was adopted. Bland-Altman figure was used to determine the differences between the two methods (*in vitro* x *in vivo*).

Results and discussion

The effects on the glycemic response to carob tablets are presented in figure 1. There were significant differences in glucose concentrations among carob tablets and the reference product in 15 ($p=0.0026$), 30 ($p<0.0001$), 45 ($p<0.0001$), and 120 ($p<0.0001$) minutes after the ingestion by the subjects. In order to calculate the glycemic load, we established a portion of 50 g for carob tablets and 10 g for carob flour (*in vitro* study). The calculated glycemic index and glycemic load of carob tablets are presented in table I.

The glycemic index of carob derived products has not been established in previous studies. The carob tablet is a product similar to chocolate in bars. The glycemic index of chocolate bars found in literature varies between 21 – 54 (Atkinson et al., 2008) The carob bar

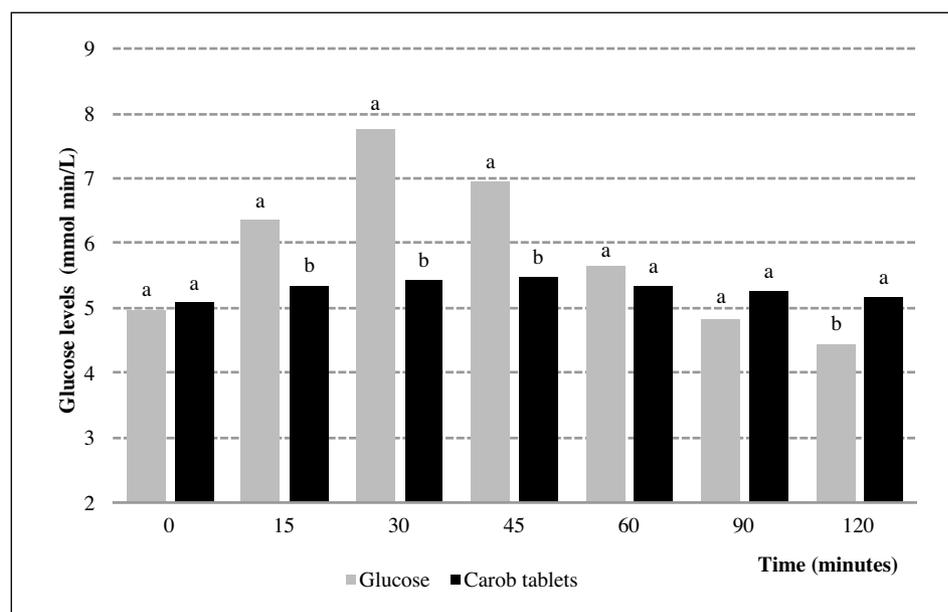


Fig. 1.—Glycemic response to glucose and carob tablets. Each value represents the mean of seven replicates. For each time, values not sharing a common letter were significantly different, $P < 0.05$.

Table I
In vivo determination of the area under the curve (AUC), glycemic index (GI) and glycemic load (GL) of carob tablets

Product	AUC	GI	GL
Glucose	114.6 ± 41.18	–	–
Carob tablets	38.23 ± 23.28	38.60 ± 23.2	10.04

Each value represents the mean of seven replicates.

proved to be a viable product to replace cocoa preparations, as obtained a glycemic index <50 and a glycemic load of 10.04.

According to Foster-Powell et al. (2002) both the reference food and test foods must contain the same amount of available carbohydrate, typically 50 or 25g. When the glucose is used as the reference, the glycemic index can be classified into low (≤ 55), moderate (56-69) or high (≥ 70). Considering the glycemic load, food can be classified into low when the glycemic load is less than or equal to 10, moderate when the glycemic load is between 11 and 19 and high when the glycemic load is greater than or equal to 20 (Capriles et al., 2009).

Low glycemic index foods promote slow postprandial blood glucose increase and gradual energy supplies to the body, while the high glycemic index foods promote rapid increase in blood glucose and immediate supply of energy (Ek et al., 2012).

Feldman et al. (1995) evaluated changes in GI following consumption of an Israeli preparation named *melawach*. This was enriched with carob, maize or lupine. The authors observed that preparations enriched with carob decreased serum glucose in diabetic patients and resulted in better glycemic control. The preparations fortified with lupine or maize showed no significant changes in glucose and insulin levels.

The results of the *in vitro* method of the glycemic index performed by the evaluation of the area under the curve, hydrolysis index, glycemic index and glycemic load are presented in table II. These results indicate that carob tablets and carob flour could be classified as low-glycemic foods and as low glycemic load foods.

In order to demonstrate the differences between the *in vitro* and the *in vivo* methods we used the Bland-Altman Scatter Plot (Fig. 2). It showed an agreement between *in vitro* and *in vivo* measurements ($R^2=0.9563$). The *in vitro* methods may estimate the biological response because the carbohydrate sensibility to digestive enzyme action is an essential factor in both *in vivo* and *in vitro* responses (Araya et al., 2002). Several studies have shown an association between glycemic index and *in vitro* available glucose (Menezes et al., 1996; Englyst et al., 1999; Parada and Aguilera, 2009).

Carob products assessed by *in vitro* hydrolysis showed different patterns of digestion; carob flour has a glycemic peak in the first 15 minutes. Carob tablets display a glycemic peak after 30 minutes of intake. Mixed ingredients in a meal may influence the response on blood glucose concentration (Gibson et al., 2011). In our study, this difference may be related to the composition of the two products, carob flour is composed only of toasted carob, and carob tablet is a product similar to chocolate, which has in its composition a significant amount of lipids. The presence of lipids may be responsible for the delayed glycemic peak of the bar.

The fiber content of two types of carob flour was analyzed. Table III shows the results for total, soluble and insoluble fiber, indicating that carob flour with different toasting degrees has similar fiber content.

The obtained results demonstrated that carob flour has a high content of dietary fiber in its composition and can be classified as a food with high fiber content, according to the Brazilian legislation (Brazil, 1998). Dietary fiber plays a significant role in the prevention

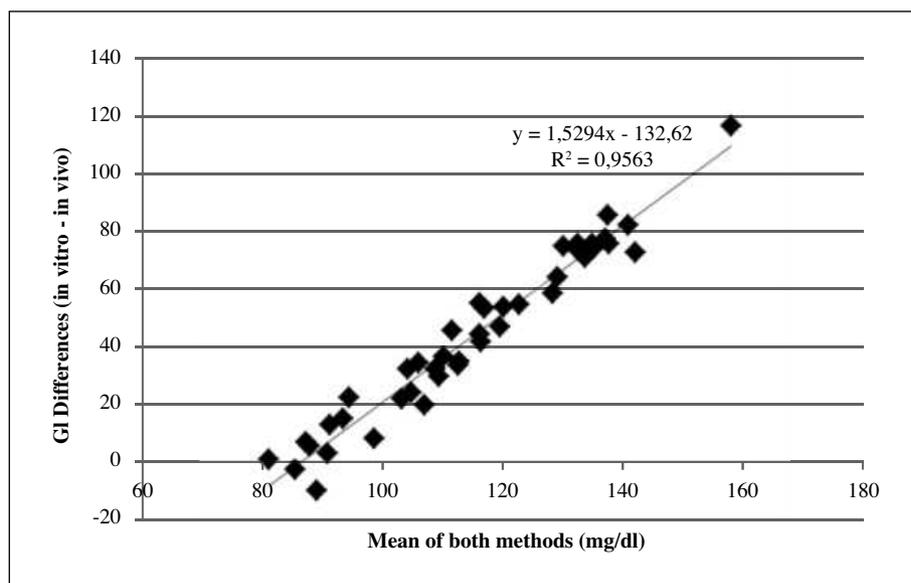


Fig. 2.—Bland and Altman Plot of the data obtained from carob tablets samples analyzed by *in vitro* and *in vivo* measurements in order to obtain the calculated glycemic index (GI). Correlation $R^2=0.9563$.

Table II
In vitro determination of the hydrolysis index (HI),
 glycemic index (GI) and glycemic load (GL)
 of carob tablets and carob flour

Product	HI	GI	GL
Carob tablets	0.69 (±0.12)	40.1 (±0.02)	10.5
Carob flour	1.73 (±0.09)	40.6 (±0.05)	10.8

Each value represents the mean of six replicates.

GI was calculated using the equation proposed by Goñi et al. (1997):
 GI = 39.71 + 0.549HI.

Table III
 Fiber content of carob flour

	LT	MT
Total fiber (%)	42.9 ± 0.68 ^a	42.6 ± 0.49 ^a
Soluble fiber (%)	2.69 ± 0.001 ^a	2.75 ± 0.001 ^a
Insoluble fiber (%)	40.0 ± 0.01 ^a	39.7 ± 0.02 ^a

LT = less toasted flour; MT = more toasted flour.

Each value represents the mean of three replicates. Means in a line with different superscripts differ (P<0.05).

of several diseases. It acts as a protective agent against cardiovascular diseases, diverticulitis, constipation, irritable colon, colon cancer and diabetes. The insoluble fraction of the fiber seems to be related to the intestinal regulation, whereas the soluble fiber is associated to the decrease of cholesterol levels and the adsorption of intestinal glucose. Foods rich in fiber have also the capacity of binding bile acids, metabolites of cholesterol, which plays an important role in the digestion and absorption of lipids in the small intestine (Gruendel et al., 2007). The locust fibers have the ability to reduce serum cholesterol and triglycerides by applying beneficial effect on metabolism of postprandial lipids (Rodríguez et al., 2006).

Our group examined (data not published) the absorption capacity and water retention by carob flour, and showed that, although the water absorption have been smaller than other products, the water holding capacity of carob fiber was higher, indicating that carob absorbed water, and this is retained by the fiber. Other products studied showed greater water absorption values, but with less water retention capacity.

The speed of absorption of carbohydrate is directly related to other dietary components such as proteins, lipids and fibers. The capacity for absorption and water-retention is directly related to the content of fibers present in the food. The water retention capacity of the fiber ingested is related to the greater volume of fecal cake, greater sense of satiety, delayed gastric emptying of carbohydrate-rich meals as well as lower glycemic response (Mishra and Monro, 2012).

The effect of soluble fiber in reducing glucose absorption speed has been attributed both to the gastric emptying delay and as a result of adsorption and in-

teraction with other nutrients, giving a lower surface of direct contact with the wall of the small intestine. The greatest resistance to diffusion through the mucosa occurs due to the viscosity of the bolus in a high fiber diet (Sartorelli and Cardoso, 2006). The insoluble fibers decrease the intestinal transit time, increase the fecal volume, make slower absorption of glucose and slow hydrolysis of starch (Souza and Menezes, 2004).

Conclusions

The results obtained in this study classify the carob tablets and carob flour as low glycemic index food and low glycemic load food. Carob flour is classified as a high fiber food, containing mainly high levels of insoluble fiber. This high content of dietary fiber makes carob an ingredient with physiological benefit effects.

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