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CHANGES IN THE ANTIOXIDATIVE ACTIVITIES OF BEAN PRODUCTS AND INTESTINAL MICROFLORA IN THE MODEL OF THE GASTROINTESTINAL TRACT "*IN VITRO*"

Małgorzata Gumienna, Kamila Goderska, Jacek Nowak, Zbigniew Czarnecki

Department of Fermentation and Biosynthesis, Institute of Food Technology of Plant Origin, The August Cieszkowski Agricultural University of Poznań, Poznań

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The aim of the research project was to determine the effect of the human intestinal microflora on changes in the active, natural biological compounds which occur in products obtained from bean seeds. In addition, the authors ascertained the "*in vitro*" microflora survivability in the applied model of the gastrointestinal tract during the digestion of these products. During the "*in vitro*" digestion process, the highest antioxidative activity was determined in the case of the instant flour and extruded products (*ca*. 46 mg Tx/g) at a relatively low total content of phenol compounds – 5.94 and 3.35 mg/g, respectively. The products subjected to the digestion process stimulated the growth of intestinal microorganisms and, at the stage of the "large intestine", their numbers ranged from 10^8 to 10^9 cfu/mL in the case of all the identified microorganisms.

INTRODUCTION

The "*in vitro*" model of digestion allows to apply all the digestion mechanisms that occur in a living organism in extracorporeal conditions. This makes it possible to verify the course of the digestion process. In order to achieve environmental conditions which exist in the human gastrointestinal tract, it is essential to ensure such processes as: digestion with pepsin and pancreatin, appropriate adjustment of the pH value, peristaltic movements, a satisfactory set of the suspension acceptors and a characteristic permeable enterocyte epithelium [Gil-Izquierdo *et al.*, 2001; Ekmekcioglu, 2002]. The settlement of the gastrointestinal tract by the appropriate intestinal microflora is also very important. It constitutes the most important and complex ecosystem made up of approximately 500 different species of microorganisms [Gawęcki & Libudzisz, 2006]. This numerous and diverse complex of microorganisms constitutes a huge catalytic potential in the human organism whose activities may both be beneficial but also constitute a very serious threat for their host. That is why it is essential to evaluate changes of selected nutrients (such as bioactive substances) and determine the survivability of the intestinal microflora in the course of the digestion process.

The objective of the presented study was to determine the activity and interactions of the human intestinal microflora with biologically active compounds and to ascertain the degree of its "*in vitro*" survivability in the applied model of the gastrointestinal tract.

MATERIALS AND METHODS

The experimental material comprised seeds of colour bean cv. Red Kidney obtained from the 2005 harvest. The following materials were used in experiments carried out in the developed model of the "*in vitro*" gastrointestinal tract: flour obtained following the comminution of bean seeds (reference sample), instant type of flour as well as products of extrusion obtained after biotechnological treatment. The instant flour was obtained from the Division of Concentrates of the Institute of Biotechnology of the Agro-Food Industry in Poznań.

The applied biotechnological processing comprised the grinding of seeds, moistening to the moisture content of about 50% and then subjecting to the bacterial fermentation process (*Lactobacillus plantarum* T-106) at the temperature of 37°C for the period of 18 h. The parameters of the extrusion process were selected on the basis of experiments carried out by Czarnecka *et al.* [1998]. The extruded products were obtained from the bean post-fermentation meal and ground maize mixed at the ratio of 1:1.

Conditions of the "*in vitro*" digestion process. The "*in vitro*" digestion was conducted in a glass bioreactor equipped in 4 inlets allowing the introduction of the pH electrode, programming of the active acidity, dosage of biochemical agents and appropriate media as well as collecting analytical samples.

Samples for the "*in vitro*" digestion process were prepared by taking 20 g of bean flour, instant flour or extruded products and dissolving them in tap water to the volume of 200 mL.

The conditions of the "digestion" process in the bioreactor were designed in such a way as to comprise the following stages of the model: the "stomach", the "small intestine" and the "large intestine" at the temperature of 37°C. The parameters of the digestion process were selected on the basis of our

Author's address for correspondence: dr inż. Małgorzata Gumienna, Institute of Food Technology of Plant Origin, Department of Fermentation and Biosynthesis, The August Cieszkowski Agricultural University of Poznań, ul. Wojska Polskiego 31, 60-624 Poznań, Poland; tel.: (48 61) 84 87 267, fax: (48 61) 84 87 314; e-mail: gumienna@au.poznan.pl

own investigations but also taking into consideration studies carried out by Aura *et al.* [1999], Gil-Izquierdo *et al*. [2001], Hoebler *et al.* [2002] and Knarreborg *et al.* [2002].

The employed intestinal microflora was collected from faeces of 3 volunteers 25 to 30 years of age. The standardization of the intestinal microflora was conducted in accordance with the methodology prepared by Knarreborg *et al.* [2002].

In order to control the influence of the conditions prevailing in the gastrointestinal tract on the growth of microorganisms, control inoculations were made 2 h after the moment of the introduction of microorganisms into the environment and at the moment of termination of the digestion process (after 21 h). The determined groups of microorganisms included: *Entrobacteriaceae* (MacConkey selective medium), *Lactobacillus* (MRS medium – agar), *Enteroccocus* (substrate – agar with kanamycin, esculin and sodium azide), *Bifidobacterium* (Garche's medium). The number of microorganisms introduced into the digestion process accounted for *ca*. 10^6 cfu/mL.

Analytical methods. During the digestion process the total polyphenol content and the antioxidation potentials were determined.

The extraction process was carried out using a 70% solution of acetone employing a single extraction of polyphenols from the samples examined.

The total polyphenol content in the extracts was determined employing the reaction with the Folin and Ciocalteu phenolic reagent according to the method developed by Singleton & Rossi [1965]. The antioxidative activity was determined against the ABTS reagent (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) according to the method described by Re *et al.* [1999]. The activity was expressed as converting into mg of Trolox, which corresponds to the force of antioxidative properties of the extract examined.

The number of live bacterial cells was determined using the Koch's plate method.

RESULTS AND DISCUSSION

Products subjected to the "digestion" process were characterised by a different initial concentration of phenolic compounds and antioxidative activity. The highest concentration of phenolic compounds as well as the highest antioxidative potential was observed in the flour obtained directly after seed comminution of colour bean (Figures 1 and 2).

Significant differences in the content of phenolic compounds as well as their antioxidative activity were found at all stages of the performed process of digestion. The highest content of phenolic compounds was recorded in the case of all products after the process of digestion carried out in the "stomach" (5.95 mg/g – flour, 6.85 mg/g – instant flour and 4.52 mg/g – extruded products). Following the stage of digestion in the "small intestine" and the "large intestine", significant reductions in the concentrations of these compounds were recorded: 2.64 mg/g – flour, 5.94 mg/g – instant flour and 3.35 mg/g – extruded products, respectively (Figure 1). However, the highest antioxidative activity was determined at the stage of digestion in the "large intestine" in which for the products obtained from colour bean, it amounted to: 26.19 mg Tx/g – flour, 47.54 mg Tx/g – instant flour and 45.80 mg Tx/g – extruded products, respectively (Figure 2).

The determined relatively high concentration of antioxidants as well as the high antioxidative potential determined at the stage of digestion in the "stomach" can be attributed to the activity of digestive enzymes. During this process, phenolic compounds are probably liberated from the glycoside flavones as a result of hydrolysis of the glycoside bond which occurs between the sugar residue and hydro cyclic ring. The above transformation leads to the development of an active aglycon which is more reactive than the glycoside form and which can be recognised as one of the more favourable changes taking place in the course of the performed biotechnological process (lactic fermentation) and "*in vitro*" digestion [Hag *et al*., 2002; Sakakibara *et al*., 2003; Grajek, 2003]. Another factor which cannot be overlooked is the action of the intestinal microflora which, due to the metabolic processes taking place, primarily, in the large intestine, can increase the antioxidative potential of the digested products [Gawęcki & Libudzisz, 2006].

The evaluation of the quantity of the intestinal microflora under conditions of the "*in vitro*" gastrointestinal tract allowed the authors to determine high survivability of all four groups of bacteria in the "*in vitro*" model conditions of the gastrointestinal tract in the course of the digestion process of products obtained from legume seeds (Table 1).

Following the performed digestion process, the authors recorded at least a ten-fold increase in the numbers of bacteria in the course of the consecutive stages of the "*in vitro*" digestion. Both bacteria from the groups of *Lactobacillus*, *Bifidobacterium*, *Enterococcus* as well as *Enterobacteriaceae* exhibited intensive growth in the presence of all products obtained from the seeds of leguminous plants. The advancing digestion process as well as the increase of the pH value of the digesta from

TABLE 1. Quantitative changes of the intestinal microflora in the course of digestion of selected products.

Microorganisms	Product (Log_{10} cfu/mL of digesta, mean ± SD)						
	Inoculum	Flour		Instant flour		Extruded products	
		[1]2h	[2]21h	[1]2h	[2]21h	[1]2h	[2]21h
Bifidobacterium	6.44±0.19	7.45±1.34	8.28±1.44	7.44±0.22	8.28±0.21	7.45±0.26	9.30±0.16
Lactobacillus	6.21±0.13	7.61±1.41	8.69±1.01	7.12±0.1	8.60±0.12	7.20±0.18	8.80±0.12
Enterococcus	6.31±0.34	7.69±1.19	9.46±0.45	7.84±1.30	9.65±0.25	7.55±0.37	9.42±0.35
Enterobacteriaceae	6.33±0.18	8.62±0.59	9.47±1.59	8.13±0.27	9.64±0.36	7.47±0.16	9.84±0.20

[1]after the period of incubation in the "small intestine" together with the intestinal microflora; [2]after the period of incubation in the "large intestine"

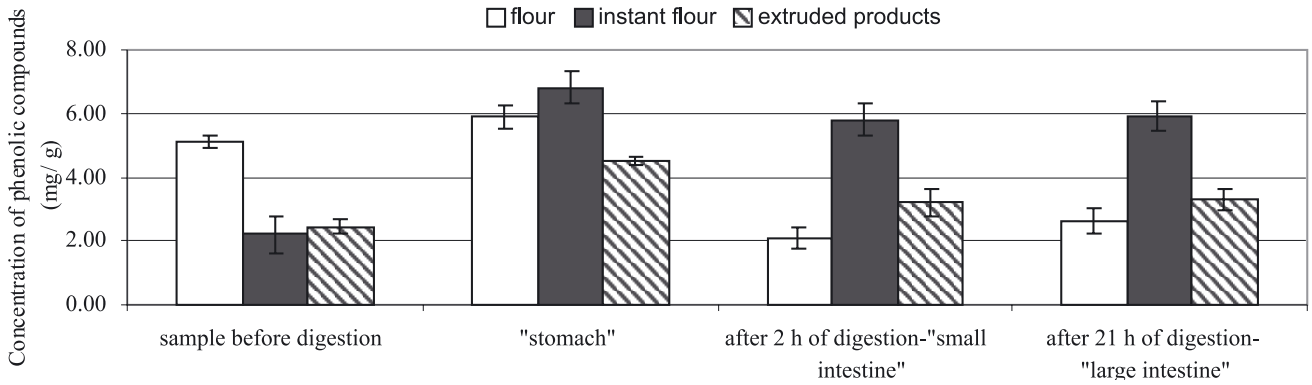

FIGURE 1. Changes in the concentration of phenolic compounds in products obtained from the colour bean of cv. Red Kidney determined at the consecutive stages of the "digestion" process.

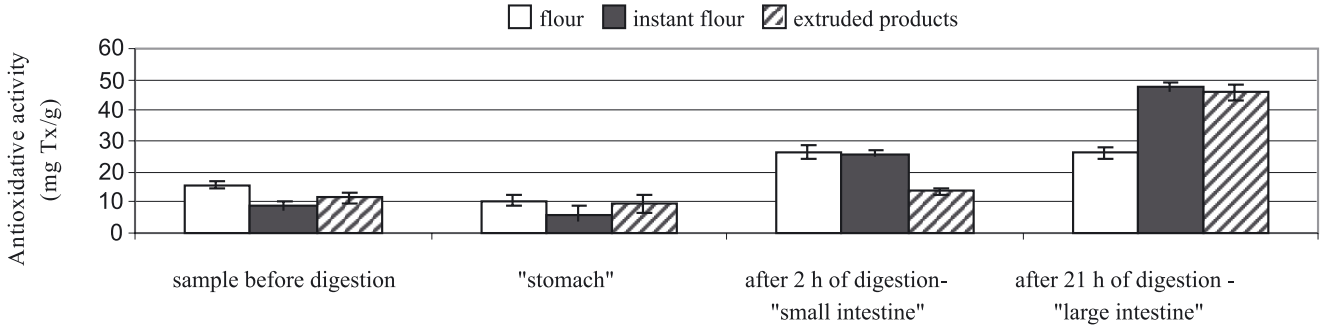

FIGURE 2. Changes in the antioxidative activity in products obtained from the colour bean of cv. Red Kidney determined at the consecutive stages of the "digestion" process.

7.4 to 8.0 did not inhibit the growth of any of the tested groups of bacteria. Following the digestion process in the "large intestine", their numbers ranged from 10^8 to 10^9 cfu/mL in the case of all the identified microorganisms. The results obtained in these experiments corroborate the theses that some physiological functions of the bacterial cell such as: the resistance to pH changes, growth temperature and availability of substrates essential for growth, exert a decisive influence on the survivability of the definite group of microorganisms in the human gastrointestinal tract [Goderska *et al.*, 2002].

CONCLUSIONS

1. The highest antioxidant activity was determined for the instant flour and extruded products at a relatively low total content of phenol compounds during digestion process at the stage of the "large intestine".
2. Products obtained from seeds of colour bean stimulated the growth of the intestinal microorganisms and following the stage of the digestion process in the "large intestine", their numbers increased from the initial value of 10^6 to the value of 10^8 -10^9 cfu/mL in the case of all the identified microorganisms.

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ZMIANY AKTYWNOŚCI ANTYOKSYDACYJNEJ PRODUKTÓW Z FASOLI ORAZ MIKROFLORY JELITOWEJ W MODELU PRZEWODU POKARMOWEGO „*IN VITRO*”

Małgorzata Gumienna, Kamila Goderska, Jacek Nowak, Zbigniew Czarnecki

Instytut Technologii Żywności Pochodzenia Roślinnego, Zakład Fermentacji i Biosyntezy, Akademia Rolnicza im. Augusta Cieszkowskiego, Poznań

W pracy określano wpływ mikroflory jelitowej człowieka na zmiany naturalnych związków biologicznie aktywnych występujących w produktach otrzymanych z nasion fasoli. Określano również przeżywalność mikroflory w zastosowanym modelu przewodu pokarmowego „*in vitro”* podczas trawienia tych produktów. W czasie procesu trawienia „*in vitro”* największą aktywność antyoksydacyjną oznaczono w przypadku mąki instant i ekstrudatów (około 46 mg Tx/g) przy relatywnie niskiej ogólnej zawartości w nich związków fenolowych odpowiednio 5,94 i 3,35 mg/g. Poddane procesowi trawienia produkty stymulowały wzrost mikroorganizmów jelitowych, i na etapie „jelita grubego” liczba ich wynosiła 10^8- 10^9 cfu/mL w przypadku wszystkich identyfikowanych mikroorganizmów.