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## DETERMINATION OF HIDDEN GLUTEN IN FOOD SUPPLEMENTS AND FOODS FOR PARTICULAR NUTRITIONAL USES FROM SERBIAN MARKET

Bojana B. Vidović<sup>1</sup>, Ivan M. Stanković<sup>1</sup>, Slađana S. Šobajić<sup>1</sup>, Nađa D. Vasiljević<sup>2</sup>, Mirjana Milanović-Stevanović<sup>3</sup>, Saša D. Janković<sup>3</sup>

<sup>1</sup>University of Belgrade, Faculty of Pharmacy, Department of Bromatology, 11000 Belgrade, Vojvode Stepe 450, Serbia

<sup>2</sup>University of Belgrade, Faculty of Medicine, Institute of Hygiene and Medical Ecology, 11000 Belgrade, Dr Subotića 8, Serbia

<sup>3</sup>Institute of Meat Hygiene and Technology, 11000 Belgrade, Kačanskog 13, Serbia

\*Corresponding author:

Phone: +381113951395

Fax: +38113972840

E-mail address: [bojana@pharmacy.bg.ac.rs](mailto:bojana@pharmacy.bg.ac.rs)

**ABSTRACT:** Coeliac disease is characterized by permanent intestinal intolerance to wheat gliadin and related prolamins from barley, rye and oats. The gluten free diet is a life long treatment for coeliac patients. Industrial starch or other ingredients used in manufacturing of dietary products can contain prolamins as impurities. The aim of this work was investigation the presence of gluten in dietary products from the national market: 30 dietary foods for infants and young children and 35 food supplements were analyzed using the Ridascreen Gliadin sandwich R5 enzyme-linked immunosorbent assay with cocktail extraction. The most of the analyzed samples (88%) contained less than limit of quantification of 5 mg/kg for gluten. Gluten content of 5-20 mg/kg was determined in 3 samples. Five of 65 samples (8%) contained mean gluten levels  $\geq 20$  mg/kg and would not be considered "gluten-free" according to the national Regulation on health safety of dietary products. The systematic control of the presence of gluten in gluten-free dietary products is very important and should be obligatory in order to protect the health of coeliac patients.

**Key words:** *coeliac disease, gluten, dietary products, food supplements, baby foods*

## INTRODUCTION

Coeliac disease (CD), also known as coeliac sprue and gluten sensitive enteropathy, is characterized by permanent intestinal intolerance to wheat gliadin (and related prolamins from barley, rye and oats), which causes damage to small bowel mucosa by an autoimmune mechanism in genetically susceptible individuals (Hill et al., 2005). Epidemiological studies have shown that CD affect approximately 0.5–1.0% of people in developed countries (Catassi and Fasano, 2008) and its prevalence is on the rise (Rubio-Tapia and Murray, 2010). Untreated CD can result in severe malnutrition, and stunted growth in children (Fasano and Catassi, 2005); gynaecological, reproductive and obstetrical dysfunctions (Smecual et al., 1996); skeletal (Blazina et al., 2010), psychiatric, neurological (Collin et al., 1991) and hepatobiliary diseases (Kaukinen et al., 2003); malignomas (Green et al., 2003); increased risk of associated autoimmune diseases (Ventura et al., 2002).

The gluten-free diet is the essential treatment for coeliac patients and requires avoidance of any food or other products containing prolamins from wheat (gliadins), rye (secalins), barley (hordeins) and oats (avenins). However, total avoidance of these prolamins is not so simple because of their presence in unexpected foodstuffs such as meat products, soups, sauces, juices and beers, as well as in food additives like flavorings, emulsifiers, colorings and preservatives that are derived from gluten-containing grains (Gregorek et al., 2006). Some research suggested that some CD patients can tolerate oats, but it also might be contaminated with wheat, barley, and/or rye during harvest, transport, and/or processing (Koerner et al., 2011). Also, industrial starch and other ingredients used in manufacturing pharmaceutical products and food supplements can contain prolamins as impurities.

In the present study, the presence of hidden gluten in a range of dietary products available on the Serbian market has been investigated.

## **MATERIAL AND METHODS**

Thirty five food supplements and thirty samples of dietary foods for infants and young children from Serbian market were analyzed using a commercially ELISA kit (RIDASCREEN® Gliadin, Art. No. R7001) produced by R-Biopharm AG, Darmstadt, Germany. This sandwich ELISA kit has a detection limit of 3 mg/kg of gluten and is based on the monoclonal antibody R5 that reacts with gliadin-fractions from wheat and corresponding prolamins from rye and barley (Valde's et al., 2003; Mendez et al., 2005). The quantification range of this assay is 5 to 80 mg/kg of gluten. The method has been endorsed by the Codex Committee on Methods of Analysis and Sampling as a type 1 method for determination of the gluten content in gluten-free foods (ALINORM 06/29/23).

### **Samples extraction and preparation**

0.25 g of the homogenized sample of dietary product was weighed into a 10 ml centrifuge tube, a Cocktail-Solution (2.5 ml) was added and the tubes were put on a vortex for 30 s. Samples were incubated for 40 min at 50 °C, then mixed with 7.5 ml of 80% ethanol and incubated for 1h in a rotary shaker at 45 turns/min. The tubes were centrifuged at 2500 x g for 10 min at room temperature and the supernatants were used for sandwich ELISA analysis. A measured aliquot of the supernatant was removed; diluted 1:12.5 (80 µl + 920 µl) and then 100 µl of this solution were used in the assay.

### **Gluten determination**

Standards and samples were added in duplicate wells on the plate and allowed to incubate for 30 min at room temperature followed by three washing steps. Enzyme conjugate was added to each well and the plate was incubated for 30 min followed by an additional three washing steps. At this point substrate and chromogen were added to each well and allowed to react for 30 min followed by the addition of stop reagent. The absorbance was read at 450 nm on a BioTek EL800 microplate reader (BioTek Instruments, Inc., Winooski, VT) and the data were analysed to determine gluten concentration.

## **RESULTS AND DISCUSSION**

According to the regulations in Serbia ("Sl.glasnik RS", No.45/2010) the maximum level of gluten in products labelled as gluten-free is 20 mg/kg. In the this study we have determined the content of gluten in thirty five samples of food supplements, produced by different manufacturers, that according to their nature should not contain gluten (Table 1). Among them, only five samples of food supplements had specifically mentioned gluten status. Therefore, these samples are not recognised as gluten-free by CD patients. The obtained results have shown that most of the analyzed food supplements have gluten content less than limit of detection (3 mg/kg) and limit of quantification (5 mg/kg) method. Gluten content of 5-20 mg/kg was determined in three food supplements, and only one analyzed sample had gluten content higher than 80 mg/kg.

Table1. Gluten content in analysed food supplements

Sample	Type of dietary supplement	Producer	Gluten content (mg/kg)
1	multiminerals and vitamins, chewable tablets	A	-
2	multivitamins, granules	B	-
3	multiminerals and vitamins, tablets	B	-
4	multivitamins with fish oil, syrup	C	-
5	vitamin A+D3, drops	D	-
6	<b>vitamin D3, capsules*</b>	E	-
7	calcium with vitamins A&D, powder	F	-
8	selenium with vitamins, capsules	B	9,8
9	coenzyme Q10 with vitamin and mineral antioxidants, powder	A	-
10	chromium, capsules	B	6,4
11	iron, capsules	B	>80
12	cod liver oil, softgels	G	-
13	fish oil, softgels	H	-
14	<b>fish oil, softgels*</b>	I	-
15	fish oil, softgels	J	-
16	fish oil, softgels	I	-
17	fish oil, <i>Ginko biloba</i> extract+ B vitamins, capsules	K	-
18	fish oil, softgels	A	-
19	glucosamine + chondroitine, tablets	K	-
20	<b>glucosamine, capsules*</b>	I	-
21	<i>Aloe vera</i> gel with glucosamine and chondroitine, drink	A	-
22	royal jelly, powder	L	-
23	royal jelly, powder	M	-
24	honey with propolis and essential oils	N	-
25	propolis and <i>Echinacea purpurea</i> extract, drops	O	13.5
26	pollen extract, tablets	P	-
27	whey protein, powder	Q	-
28	<b>whey protein, powder*</b>	R	-
29	proteins, powder	R	-
30	colostrum, tablets	S	-
31	lactoferrin, capsules	T	-
32	<b>collagen with vitamin C, tablets*</b>	U	-
33	<i>Ganoderma lucidum</i> extract+ <i>Perna Canaliculus</i> extract, syrup	V	-
34	chitosan, capsules	F	-
35	<i>Aloe vera</i> gel, drink	A	-

Note: “-“= gluten content < 5 mg/kg.

\*= labelled gluten status.

Table 2 shows the results for the dietary foods for infants and young children samples analyzed for the presence of gluten in this study. In four of five of samples of instant baby foods containing only naturally gluten-free ingredients the quantity of gluten have exceeded 20 mg/kg. Considering the high amount of gliadin in these samples, gluten contamination possibly occurred during manufacturing of these products.

Table2. Gluten content in dietary foods for infants and young children

Groups of products	Produce r	Numbe r	Gluten content (mg/kg)
<b>baby porridge</b>	A	25	-
<b>instant baby food</b>	B	5	
rice cereal with prebiotics			>80
rice and corn with milk			>80
rice and corn cereals with milk and fruits			>80
cereal with vanilla rich vitamins and minerals			>80
skim milk cereal with rice rich in vitamins and minerals			-

Note: “-“= gluten content < 5 mg/kg

## CONCLUSIONS

The most of the naturally gluten-free food supplements analyzed in our study, although not bearing the “gluten-free” label, can be safely consumed by coeliac patients. However, our finding of possible contamination with gluten of the products, claimed to be gluten-free, especially grain-based products, suggested that systematic control of gluten-free dietary products should be obligatory. Thus, the printing of information “gluten-free” on the labels of dietary products exclusively on the basis of ingredients without any laboratory analyses should not be allowed.

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