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Effects of gluten-free breads, with varying functional supplements, on the biochemical parameters and antioxidant status of rat serum

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ABSTRACT

This paper examines the effects of gluten-free bread enriched with functional ingredients (milk powder, poppy, sunflower and pumpkin seeds, egg yolk, carum, hazel nuts and amaranth) on the morphological and biochemical parameters and antioxidant status of rats serum. Rats were provided test diets – gluten-free breads and water *ad libitum*. After 14 days, the animals were weighed and killed. A hazel nut-amaranth bread diet significantly increased the level of thrombocytes when compared to control bread. A mixed bread diet significantly decreased cholesterol levels in rats. All fortified breads decreased triglyceride levels and alanine transaminase activity and caused an increase in antiradical activity of the serum. In rats fed with poppy-milk bread, milk-seed bread and mixed bread, a marked decrease in superoxide dismutase activity was found. Enriched breads reduced the levels of triglyceride and improved the antiradical properties of serum, although the physiological relevance of this needs to be confirmed by human studies.

1. Introduction

Bread is one of the most popular staple foods in the world. As a result of its nutritive value, low price, and simplicity of usage, it has become the basis of all civilizations' diets. Bread consumption provides energy (mainly from starch) and delivers dietary fiber, proteins and a wide range of vitamins and minerals (Nanditha & Prabhasankar, 2009). Bread is mainly produced from wheat and rye flours containing, *inter alia*, prolamins, and a protein fraction (determined as gluten) responsible for adequate structure and quality. However, there is a group of people for whom consumption of prolamins – gliadins of wheat, secalins of rye, hordeins of barley and avenins of oats – causes disorders, such as: coeliac disease, allergy, and non-celiac gluten sensitivity. In addition, their consumption leads to damage to the mucosa of the small intestine. It contributes to impaired absorption and, as a consequence, to malnutrition and the occurrence of many systemic complications (such as anemia, rickets, osteoporosis, deficit of body weight) (Hamer, 2005). A necessary precondition in the preparation of food for this group of consumers is to develop a product in such a way that the harmful factor is eliminated, while adequate value is preserved. A problem arises from the fact that, frequently, such an approach leads to a reduction in the nutritive value of the product. In the case of gluten-free foodstuffs, elimination of a raw material causing gluten intolerance in the final product (wheat, rye, barley or oats), leads to a considerable reduction of the levels of dietary fiber, vitamins B and minerals (magnesium, zinc, iron, copper).

According to the needs of consumers, optional ingredients can be added to produce special and novel bread with enhanced nutritional and nutraceutical quality and organoleptic characteristics (Balestra, Cocci, Pinnavaia, & Romani, 2011; Gambuś, Gambuś, & Sabat, 2002; Gambuś et al., 2009; Gawlik-Dziki et al., 2013; Mariotti, Lucisano, Ambrogina Pagani, & Ng, 2009; Nanditha & Prabhasankar, 2009; Świeca, Gawlik-Dziki, Dziki, Baraniak, & Czyz, 2013). Also, in the case of gluten-free bread, enrichment is closely linked with the incorporation of functional ingredients that may influence life quality and wellness (Dwyer et al., 2014). The introduction of new gluten-free products to the market requires wide-ranging studies of the safety of diets and potential positive and/or negative effects of functional food components.

The gluten-free bread in this study was supplemented with some commonly used ingredients that are characterized by well documented bioactivity *in vitro*. These are excellent sources of selected macro- and microelements (e.g., milk, poppy – Ca; carum – Fe, Cu; amaranth – Fe, Mg) (Gelderblom et al., 2013; Suliburska, Krejpcio, Reguła, & Grochowicz, 2013). Additionally, seeds and nuts used for bread enrichment are known to contain large amounts of

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bioactive components, such as phenolics, essential oils, unsaturated fatty acids, fiber, vitamins (Bozan & Temelli, 2008). Most of them exhibit prohealth properties, including antioxidant, anti-inflammatory, anticancer activities, that are usually positively correlated with phenolic levels (Liu, 2013). There have been many investigations on reducing LDL cholesterol and triglyceride concentrations and/or on improving antioxidant status in rats fed diets high in polyphenols (Hsu & Yen, 2007; Mildner-Szkudlarz & Bajerska, 2013). However, very little attention has been focussed on the cereal products which are consumed daily and which may be involved in the prevention of diet-related diseases.

Therefore, the present study was designed to investigate the safety and potential use of selected functional supplements (in commonly found combinations) for fortification of gluten-free bread. The effect of these fortified breads on the blood morphological and biochemical parameters and antioxidant status of Wistar rats was assessed as the main objective of this study.

2. Materials and methods

2.1. Chemicals

Ferrozine (PubChem CID:23662871), ABTS (PubChem CID:5815211 2), α-amylase, pancreatin, pepsin, bile extract, Folin–Ciocalteu reagent, ammoniumthiocyanate (PubChem CID:15666) and potassium hexacyanoferrate (III) were purchased from Sigma–Aldrich (Poznan, Poland). All other chemicals were of analytical grade.

2.2. Bread preparation

Breads were produced from rice flour, potato flour and corn starch (Glutenex Company, Poland), rapeseed oil (Kruszwica, Poland), salt (Solino, Poland), fresh yeast (*Saccharomyces cerevisiae*) (Lesaffre, Poland), and saccharose (Pfeifer & Langen, Poland). To enrich the product, natural components commonly available on the market were used: milk powder (SM Mlekovita, Poland), poppy seeds (VOG, Poland), sunflower seeds (VOG, Poland), flax seeds (Bio Planet, Poland), pumpkin seeds (VOG, Poland), egg yolk (Ovovita, Poland), carum (KOTÁNYI, Poland), hazel nuts (VOG, Poland), amaranth (Bio Planet, Poland) and combinations (PMB-poppy-milk bread; CB-carum bread; HAB-hazel nuts-amaranth bread; MSB-milk-seeds bread; ECB-egg yolk-carum bread; MB-mixed bread) (Table 1). White bread was used as the control bread – WB without supplements. Breads were baked using two-phase dough fermentation with a starter at 180 °C for 45 min. After baking, breads were dried and homogenized in an electric grinder.

Table 1
Breads composition.

Ingredients (%)	Breads						
	WB	PMB	CB	HAB	MSB	ECB	MB
Rice flour	20.7	19.7	17.2	17.7	17.2	17.4	16.6
Corn starch	10.4	9.9	8.6	8.8	8.6	8.7	8.3
Potato flour	20.7	14.3	10.8	11.1	10.8	10.9	10.4
Yeast	2.6	2.5	2.2	2.2	2.2	2.2	2.1
Sacharose	3.1	3.0	2.6	2.7	2.6	2.6	2.5
NaCl	0.5	0.5	0.4	0.4	0.4	0.4	0.4
Rapeseed oil	0.5	0.5	0.4	0.4	0.4	0.4	0.4
Milk powder	0.0	3.0	0.0	0.0	2.6	0.0	2.5
Amaranth flour	0.0	0.0	2.6	2.7	2.6	2.6	2.5
Flax	0.0	0.0	2.2	2.2	2.2	2.2	2.1
Sunflower seeds	0.0	0.0	2.2	2.2	1.7	2.2	2.1
Pumpkin seeds	0.0	0.0	2.2	2.2	2.2	2.2	2.1
Hazel nuts	0.0	0.0	1.7	2.7	1.7	2.2	2.1
Poppy	0.0	7.4	0.0	2.2	2.6	0.0	4.1
Egg yolk	0.0	0.0	0.4	0.0	0.0	0.9	0.4
Carum	0.0	0.0	4.3	0.0	0.0	2.6	0.0
Water	41.5	39.4	42.2	42.5	42.2	42.6	41.5

WB – white bread; PMB – poppy-milk bread; CB – carum bread; HAB – hazel nuts-amaranth bread; MSB-milk-seeds bread; ECB – egg yolk-carum bread; MB – mixed bread.

2.3. Animals and diets

Studies were conducted for 14 days on 42 growing (around 6-week-old) male rats of the albino Wistar strain with a mean body weight of 232.5 g ± 26.6. The experiment was performed with the agreement of the local bioethics committee (approval No. 888/11).

Animals were kept in individual metabolic cages in a room with natural lighting, at a temperature of 19–22 °C and relative humidity of 55–60%. The food consumption was recorded daily and the animals were weighed once a week. In the adaptation period, the animals consumed a pelleted maintenance feed for laboratory animals: Labofeed B. After a 3-day adaptation period, the animals were fed test diets (breads – Table 1) and water *ad libitum*. At the completion of the feeding period (2 weeks) on the last day of the experiment, after 12 h of fasting, the animals were anesthetized with a sodium thiopental injection (40 mg/kg body weight) and killed by cardiac puncture.

2.4. Gastrointestinal digestion in vitro

For preparation of a potentially bioavailable fraction of bread extracts, simulated digestion was performed (Świeca, Baraniak, & Gawlik-Dziki, 2013). Simulated saliva solution was prepared by dissolving 2.38 g of Na_2HPO_4, 0.19 g of KH_2PO_4, 8 g of NaCl, and 100 mg of mucin in 1 L of distilled water. The solution was adjusted to pH = 6.75 and α-amylase (E.C. 3.2.1.1.) was added to obtain 200 U per ml of enzyme activity. For the gastric digestion, a solution of 300 U/ml of pepsin (from porcine stomach mucosa, pepsin A, (EC3.4.23.1) in 0.03 M NaCl, pH = 1.2, was prepared. Further, simulated intestinal juice was prepared by dissolving 0.05 g of pancreatin (activity equivalent 4 × USP) and 0.3 g of bile extract in 35 ml of 0.1 M $NaHCO_3$. The bread samples were subjected to simulated gastrointestinal digestion as follows: 1 g of powdered sample was homogenized in a stomacher laboratory blender for 1 min to simulate mastication with the presence of 15 ml of simulated salivary fluid, and, subsequently, the samples were shaken for 10 min at 37 °C. The samples were adjusted to pH = 1.2 using 5 M HCl, and, subsequently, 15 ml of simulated gastric fluid were added. The samples were shaken for 60 min at 37 °C. After digestion with the gastric fluid, the samples were adjusted to pH = 6 with 0.1 M of $NaHCO_3$ and then 15 ml of a mixture of bile extract and pancreatin were added. The extracts were adjusted to pH = 7 with 1 M NaOH and finally 5 ml of 120 mM NaCl and 5 ml of mM KCl were added to each sample. The prepared samples were subjected to *in vitro* digestion for 120 min, at 37 °C in the darkness. After that, samples were centrifuged and supernatants were used for further analysis.

2.5. Analysis of blood morphological and biochemical parameters

The blood was collected by cardiac puncture in tubes with heparin sodium to obtain whole blood for morphological tests and in serum-separated tubes for biochemical parameters. The coagulated blood was left to clot at room temperature for 30 min, and then it was centrifuged for 15 min at 3600×*g*. The following morphological parameters were determined: WBC – white blood cells, MCV – mean corpuscular volume, MCH – mean corpuscular hemoglobin, MCHC – mean corpuscular hemoglobin concentration, LYM – lymphocytes; PDW – platelet distribution width, RDW – red blood cell distribution width, MPV – mean platelet volume, as well