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ASSESSING THE ANTIOXIDANT PROPERTIES OF SOME FUNCTIONAL FOODS, FORMULATED WITH RED AND BLACK RICE

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Abstract

Functional foods such as yoghurt made with red or black rice, previously developed, exhibit in vitro an antiproliferative effect on the colorectal cancer cell line. In this context, these studies aimed to assess the antioxidant properties of these types of bioproducts, to establish if this behaviour is due to their antioxidant properties. The postbiotics obtained from these functional foods were analysed by chemiluminescence, and the resulting antioxidant activities were compared with normal yoghurt used as a control. The results revealed that the functional yoghurts with 2.5% fat, containing red or black rice, exhibited high antioxidant activity. In conclusion, the antitumour effect exhibited on the human colorectal tumour cell line in vitro, as shown in previous studies, may also be attributed to the superior antioxidant activity of the new functional foods formulated with black or red rice.

Key words: *antioxidant properties, black rice, functional foods, red rice.*

INTRODUCTION

Functional foods containing prebiotics and probiotics have gained increasing importance recently because the probiotic microorganisms in these foods can balance the microbiome present in the digestive system. The digestive microbiome, in turn, modulates processes occurring in the central nervous system through bidirectional biochemical signalling between the gastrointestinal tract and the central nervous system (Chao et al., 2020). Current research has highlighted that functional foods with prebiotics and probiotics play an essential role in modulating processes that occur at the level of the gut-brain axis (Chao et al., 2020). In this context, prebiotics from plant sources, simple probiotics, and consortia of probiotic microorganisms are being intensely studied for their beneficial impact (Radu et al., 2016; Radu et al., 2017[abc]), and fibres from cereals can represent potential prebiotics sources for the microbiota. Studies have highlighted fibres' beneficial role in human nutrition, especially in reducing the risk of obesity and certain chronic diseases such as cardiovascular diseases, type II diabetes, and colorectal cancer (Gouseti et al., 2019). Dietary fibres, primarily derived from plant-based foods, consist of carbohydrates that form the cell walls of plants (Zaharie et al., 2022; Radu et al., 2010). Among the beneficial effects of soluble fibres introduced into human nutrition are improved blood glucose control, reduced cholesterol, and decreased starch digestibility (Botticella et al., 2021; Alahmari, 2024). Sompong et al. (2011) reported that varieties of black and red rice from China contain fibre levels ranging from 3.41-4.08 g/ 100 g of grains for black rice and 2.5-4.01 g/ 100 g of grains for red rice. Priya et al. (2019), in studies on red and black rice from India, reported that these two types of rice can contain between 0.28-0.61 g of fibre/100 g of red rice and 0.3-0.06 g/100 g of black rice, making them suitable for the development of new potential foods. Furthermore, the malts used in the beer industry could represent potential raw materials for developing functional foods with prebiotic content. Barley malt, widely used in the food industry, can contain up to 8 g of fiber/100 g of malted grains (Teixeira et al., 2018). All these aspects have previously enabled the creation of potential functional foods containing consortia of probiotic microorganisms and prebiotics based on red rice, black rice, or oat malt, which have been reported to have antitumor properties (Ciric et al., 2023).

Previous studies have shown that postbiotics resulting from newly developed functional foods contain polyphenols (Ciric et al., 2023); for this reason, the purpose of the present study was to evaluate the antioxidant activity of postbiotics from the foods that scored highest in organoleptic analyses and that meet the qualitative standards required for the type of functional food developed.

MATERIALS AND METHODS

Seven postbiotics derived from two types of functional foods obtained in previous studies (Ciric et al., 2023), with a consortium of probiotic microorganisms (*Lactobacillus acidophilus, Bifidobacterium animalis, Streptococcus salivarius, Lactobacillus delbrueckii*), and prebiotics from cereals such as red rice, black rice, or barley malt (Ciric et al., 2023), were analysed for their antioxidant activity, as follows: I) postbiotics obtained from functional foods in the form of low-fat yoghurt (0.8% fat), labelled as follow: U-L-FM (postbiotics obtained from low-fat yoghurt, without added prebiotics); 1-L-FM (postbiotics obtained from low-fat yoghurt containing prebiotics from black rice); 2-L-FM (postbiotics obtained from low-fat yoghurt containing prebiotics from red rice) and II) postbiotics obtained from functional foods in the form of full-fat yoghurt (2.5% fat), labelled as follow: U-FM (postbiotics obtained from yoghurt without added prebiotics); 1-FM (postbiotics obtained from yoghurt containing prebiotics from black rice); 2-FM (postbiotics obtained from yoghurt containing prebiotics from red rice); 3-FM (postbiotics obtained from yoghurt containing prebiotics from barley malt).

The postbiotics were obtained through centrifugation at 6000 rpm (centrifuge Hettich EBA 200S, Andreas Hettich GmbH & KG Tuttlingen, Germany). The supernatant that resulted from each bioproduct was successively filtered through 0.45 μm and 0.2 μm membranes to obtain bioproducts (postbiotics) free of bacterial load. The sterile postbiotics obtained in this manner were used in the presented studies regarding antioxidant activities. The antioxidant activity was determined by chemiluminescence, according to the methodology presented by Zaharie and collaborators (Zaharie et al., 2022), using a Glomax 20/20 chemiluminometer, E5321-Promega. The EC50 value (EC50 represent the concentration at which a bioproduct or substance shows half of its maximum activity) was evaluated using the AAT Bioquest programme (AAT Bioquest, Inc. 2025), dedicated to nonlinear bioprocesses, with equation (1), where the mathematical model approximates the values of a, b, c, and d.

$$Y = d + \frac{a - d}{1 + \left(\frac{X}{c}\right)^{b}} \qquad (1)$$

where:

a, d = coefficients predicted by math programme;

c = EC50;

b = the Hill coefficient;

X = the concentration of the substance under analysis.

RESULTS AND DISCUSSIONS

Studies performed on the postbiotics obtained from low-fat yoghurt have shown that in the case of the control sample (0.8% lipids, without added prebiotics), the corresponding postbiotic exhibits antioxidant properties at all concentrations tested (Figure 1a, b). The maximum antioxidant activity obtained for this control sample with low lipid content is 71.8% (Figure 1a), and the EC50 value determined for this biopreparation is 2.56 μL/mL (Figure 1b). The postbiotics derived from the bioproducts with 0.8% lipids and prebiotics like black rice or red rice show a different behaviour compared to the control sample. Thus, the postbiotic obtained from the bioproduct containing black rice exhibits a prooxidant character at high concentrations (determinations performed at 5 seconds). However, at lower postbiotic concentrations, or at concentrations equal to or below 6.25 µL/mL in the mixture used for evaluating antioxidant activity by chemiluminescence, the system displays antioxidant properties (Figure 2a). In this case, the antioxidant activity of the corresponding postbiotic (sample 1-L-FM) increases inversely proportional to the postbiotic concentration in the medium. The maximum antioxidant activity obtained for this postbiotic is 56.91% at c = 1.56 µL/mL (Figure 2a), and the EC50 value determined for the postbiotic derived from the 1-L-FM bioproduct is 76.65 µL/mL (Figure 2b).

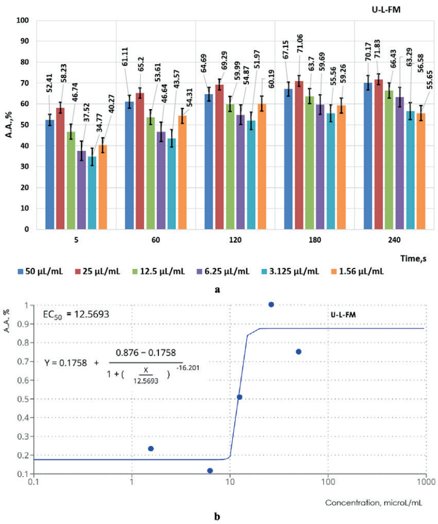

Figure 1. Antioxidant properties of functional food: a) postbiotic derived from the sample U-L-FM; b) EC50 predicted value for postbiotic derived from the sample U-L-FM

For the postbiotic obtained from the bioproduct with 0.8% lipids and red rice (bioproduct 2-L-FM), the behaviour is similar. The system becomes an antioxidant for postbiotic concentrations in the medium equal or situated below 12.50 µL/mL (Figure 3a). As in the previous case, the antioxidant activity increases inversely proportional to the postbiotic concentration in the medium. In the case of 2-L-FM bioproduct, the maximum antioxidant activity achieved is 52.86% at c = 12.50 µL/mL, and the EC50 value determined is 13.65 µL/mL (Figure 3b).

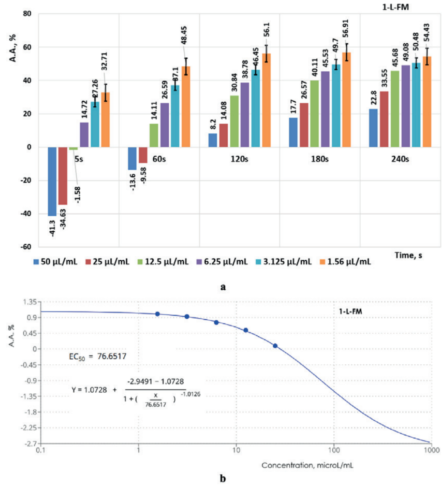

Figure 2. Antioxidant properties of functional food: a) postbiotic derived from the sample 1-L-FM; b) EC50 predicted value for postbiotic derived from the sample 1-L-FM

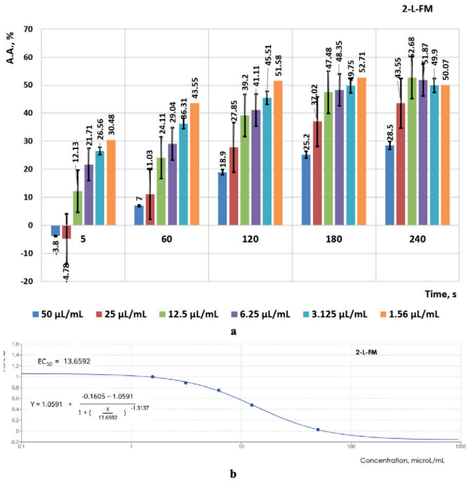

Figure 3. Antioxidant properties of functional food: a) postbiotic derived from the sample 2-L-FM; b) EC50 predicted value for postbiotic derived from the sample 2-L-FM

In the case of postbiotics derived from yoghurts with a normal lipid content (2.5%), the results obtained from the chemiluminescence study indicate a behaviour different from that of classic antioxidant systems, where the maximum antioxidant activity is achieved immediately after the addition of the antioxidant reagent to the system. The postbiotic derived from the control sample U-FM (which contains 2.5% lipids and has no added prebiotics) exhibits an antioxidant effect across the entire concentration range studied (Figure 4a). In this case, the antioxidant activity increases as the concentration of postbiotic in the medium increases and, correspondingly, with the duration of exposure. The maximum antioxidant activity obtained is 82.9%, is reached at a postbiotic concentration in the medium of 50 µL/mL after 240 seconds (Figure 4a); for this system, EC50 = 164.46 µL/mL (Figure 4b).

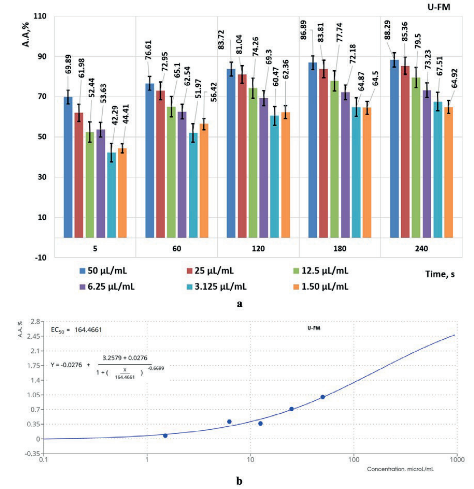

Figure 4. Antioxidant properties of functional food:
a) postbiotic derived from the sample UFM;
b) EC50 predicted value for postbiotic derived from the sample U-FM

For the postbiotic derived from the sample with 2.5% lipids and black rice (1-FM), it is observed that immediately after the addition of the postbiotic to the medium, the system exhibits prooxidant properties (measurements taken at 5 seconds), and later, at t=120 seconds, it becomes antioxidant (Figure 5a). The maximum antioxidant activity obtained for the postbiotic resulting from the 1-FM sample is 55.34%, is reached at a postbiotic concentration in the system of 3.125 µL/mL. In this case, the antioxidant activity increases inversely proportional to the concentration of postbiotics in the system and directly proportional to the exposure time. For the postbiotic derived from the 1-FM sample, EC50 = 6.37 µL/mL (Figure 5b).

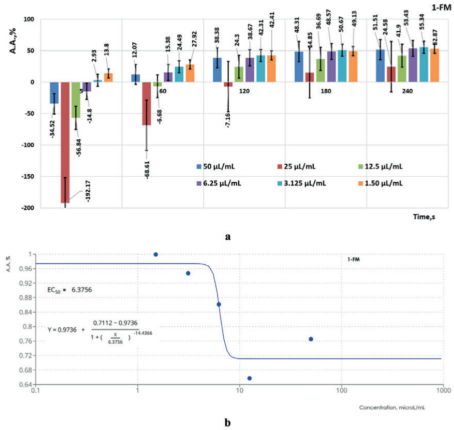

Figure 5. Antioxidant properties of functional food:
a) postbiotic derived from the sample 1-FM;
b) EC50 predicted value for postbiotic derived from the sample 1-FM

Studies performed with the postbiotic derived from the postbiotic derived from yoghurt with 2.5% lipids and red rice (2-FM) revealed a behaviour similar to that of the biopreparation with black rice. In this case, systems containing postbiotic additions at concentrations ranging between 50 and 6.25 µL/mL exhibit prooxidant characteristics after 5 seconds (Figure 6a).
After 120 seconds, the system becomes antioxidant over the entire concentration range studied. The maximum antioxidant activity obtained for the postbiotic resulting from the 2-FM biopreparation is 56.29%, corresponding to a concentration of 3.12 µL/mL (Figure 6a). For this biopreparation, EC50 = 5.85 µL/mL (Figure 6b). Again, in this case, the antioxidant activity increases inversely proportional to the postbiotic concentration in the medium.
Studies performed with the postbiotic resulting from the postbiotic resulting from yoghurt with 2.5% lipids and prebiotics from barley malt (3-FM) showed that, in the presence of this postbiotic, the system is generally prooxidant. However, for concentrations ranging between (6.25-1.56) µL/mL, from t=180 seconds, the system becomes antioxidant (Figure 7a). In this case as well, the antioxidant activity increases inversely proportional to the concentration and directly proportional to time (Figure 7a). The predictive model generated, for the postbiotic derived from 3-FM, an EC50 value of 1013.80 µL/mL (Figure 7b).

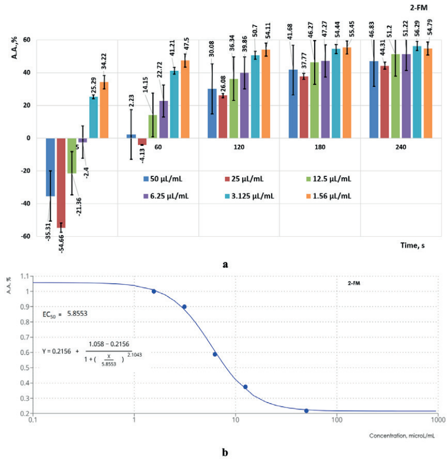

Figure 6. Antioxidant properties of functional food:
a) postbiotic derived from the sample 2-FM;
b) EC50 predicted value for postbiotic derived from the sample 2-FM

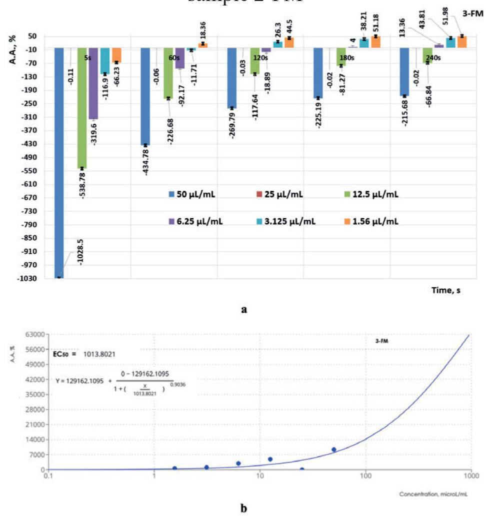

Figure 7. Antioxidant properties of functional food:
a) postbiotic derived from the sample 3-FM;
b) EC50 predicted value for postbiotic derived from the sample 3-FM

The atypical behaviour of the studied postbiotics concerning antioxidant activity is based on two main factors: 1) The determination methodology used – the chemiluminescence system can influence measurements, given that the antioxidant activity of compounds may vary depending on environmental conditions; 2) The composition of the postbiotics – the analysed postbiotics most likely contain bound polyphenolic compounds, which modify their behaviour over time under the influence of the specific environment used in the chemiluminescence studies.

In the first phase of the process, the system is prooxidant because, in a chemiluminescence matrix used, the bounded polyphenol molecules become free, a process that occurs gradually (approximately 120 s). As these molecules are released, the system acquires antioxidant characteristics (Ghazemadeh et al., 2018). Martinez-Gomez and collaborators (2020) explain the antioxidant activity of polyphenolic compounds through reaction (2), after which semiquinones are formed, which, in the final stage, transform into 1,4-benzoquinone (Figure 8).

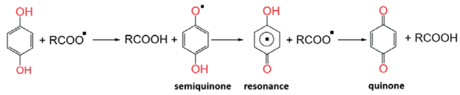

Figure 8 Antioxidant mechanism of polyphenols (adapted from Martinez Gomez et al., 2020)

The antioxidant effect of the food bioproducts without prebiotics (U-L-FM; UFM) is due to the presence in the postbiotics of microbial metabolites produced by microorganisms *Lactobacillus* sp. type (Tzang et al., 2018; Wang et al., 2017) or *Bifidobacterium* sp. (Averina et al., 2021). These compounds can be milk-derived peptides (caseokinins, lactotripeptides), bacteriocins biosynthesised by the probiotic bacteria (bifidocin), antioxidant enzymes such as superoxide dismutase and/or catalase, or exopolysaccharides synthesised by the probiotic bacteria (Tzang et al., 2018; Wang et al., 2017; Averina et al., 2021). In the case of postbiotics derived from food bioproducts containing cereal-based prebiotics, the microorganisms such *as Bifidobacterium* sp. type may transform large polyphenols molecules into polyphenolic compounds with small molecules with a stronger antioxidant character, such as ferulic acid and caffeic acid (Amaretti et al., 2013). Black and red rice contain anthocyanins and proanthocyanidins; these compounds, under the action of probiotic bacteria, are converted into smaller molecules with increased antioxidant activity (Jun et al., 2011), such as rutin, chlorogenic acid, apigenin, vanillic acid, p-coumaric acid, and

quercetin (Sompong et al., 2011; Wongsa, 2020; Kusumawardani & Luangsakul, 2024).
In the black rice, the presence of the anthocyanins compounds with antioxidant properties has been highlighted, like cyanidin-3-glucoside, cyanidin-3-rutinoside, peonidin-3-glucoside, and cyanidin-3,5-diglucoside (Ou et al., 2013; Wongsa, 2020), cyanidin-3-glucoside, pelargonidin-3-glucoside, and malvidin-3-glucoside (Walter & Marchesan, 2011; Ciulu et al., 2008; Ghasemzadeh al., 2018; Prya et al., 2019; Wongsa, 2020; Hanifa et al., 2020).
Baptista and collaborators reported in their studies that red rice contains tocopherols (α-tocopherol; γ-tocopherol), with a total concentration reaching 0.89 mg/100 g of rice grains (Baptista et al., 2024).
Furthermore, cell proliferation tests performed on various standardised cell lines demonstrated that, *in vitro*, alcoholic extracts of red rice exhibit antiproliferative effects on breast cancer (MCF-7), lung carcinoma (NCI-H460), cervical carcinoma (HeLa), and liver carcinoma (HepG2) cell lines (Baptista et al., 2024).
The same alcoholic extracts derived from red rice exhibits antimicrobial effects against pathogenic microorganisms such as *E. coli, K. pneumoniae, M. morganii, P. mirabilis, and P. aeruginosa*, the tested bioproduct showing a minimum inhibitory concentration (MIC) value > 20 mg/mL (Baptista et al., 2024).
Regarding the antioxidant effects observed for the postbiotics derived from biopreparations containing prebiotics from barley malt, these are attributed to the presence of polyphenolic compounds and vitamin E in malted barley grains, as demonstrated by Dabina Bicka (Dabina Bicka, 2011) in her studies. According to this research, maltified barley grains can contain up to 3.4 mg GAE/g, and the vitamin E content can reach up to 41.8 mg/kg (Dabina Bicka, 2011).
Gąsior and collaborators, in the studies performed on liquid samples obtained from the fermentation of barley malt with yeasts (samples taken from the beer manufacturing process), reported a polyphenol content ranging between 176 and 922 mg GAE/L, data also confirmed by studies conducted by Shopska and collaborators (Gąsior et al., 2020; Shopska et al., 2021). Leitao and collaborators reported that barley malt contains polyphenolic compounds such as protocatechuic acid, procyanidins, p-hydroxybenzoic acid, catechin, chlorogenic acid, vanillic acid, caffeic acid, epicatechin, p-coumaric acid, ferulic acid, and sinapic acid (Leitao et al., 2012).
Analysis of the EC50 values obtained in our studies reveals that the functional food coded 2-FM, obtained from milk with 2.5% lipids, fermented with a consortium of probiotic microorganisms in the presence of prebiotics from red rice, exhibits the highest antioxidant activity (EC50 = 5.85 µL/mL).
The second place is occupied by bioproduct coded 1-FM (EC50 = 6.37 µL/mL), which contains prebiotic fibres from black rice. The third place is the bioproduct U-L-FM (yoghurt without prebiotics, obtained from milk with 0.8% lipids).
Subsequently, in order of increasing EC50 values, are the bioproducts: 1-L-FM (EC50 = 76.65 µL/mL); U-FM (EC50 = 164.46 µL/mL); and finally, 3-FM, with EC50 = 1013.8 µL/mL (Figure 9).

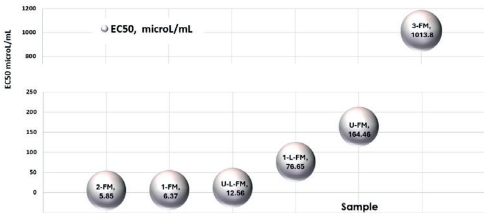

Figure 8. Variation of EC50 value for analysed postbiotics

The antioxidant activity of postbiotics derived from the 7 functional foods obtained varies in the following order:

2-FM>1-FM>U-L-FM>U-FM>3-FM
◄ **Increase antioxidant activity**

The best results are obtained for the variants made with milk containing normal lipid content and additions of red rice and/or black rice.
The best antioxidant properties are obtained for the variants made with milk containing normal lipid content and additions of red rice and/or black rice.

CONCLUSIONS

The study of the antioxidant activities of functional foods obtained from milk with a lipid content between 0.8% and 2.5%, consortia of probiotic microorganisms, and additions of cereal (black rice, red rice, or barley malt) showed that the most effective products in terms of antioxidant activity are those obtained from milk with 2.5% lipids (normal lipid content), in the presence of red or black rice, and fermented with a consortium of probiotic microorganisms containing *Lactobacillus acidophilus, Bifidobacterium animalis Streptococcus salivarius, and Lactobacillus delbrueckii*.

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