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EVALUATION OF GLUTEN-FREE BARS MADE WITH HOUSE CRICKET (*ACHETA DOMESTICUS*) POWDER

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ABSTRACT

The use of insects is a modern trend in the food industry with a positive impact on ecology and sustainability. Not only the popularity of eating whole insects is growing among consumers, but also products with an insect component are preferred more. It is also used in foods for special nutrition. Gluten-free bar made with cricket powder can represent a functional food stuff. The study shows that the addition of insects can increase the Total Polyphenol Content (24.78 mg GAE/ 100 g to 44.68 mg GAE/ 100 g) ($p < 0.05$). There is also an increase in antioxidant activity expressed as scavenging activity (%) for ABTS from 7.33 to 50.17, for DPPH 4.10 to 33.88 ($p < 0.05$). The influence of cricket powder was also recorded in the moisture content of the product, when the increased addition of insects led to a decrease in moisture content (R=0.89). Sensory evaluation showed overall acceptability for these bars. Texture, taste, appearance, aroma and overall pleasantness were compared. Although the protein bars without cricket content (Ctrl) had the highest overall pleasantness rating, it differed statistically significantly ($p < 0.05$) only from the sample with the highest cricket powder content (B25I).

Keywords: edible insect, sensory analysis, antioxidant activity, cricket

INTRODUCTION

Cereals, primarily wheat, rye, and barley, form an integral part of human diet, but for some people their consumption causes a disease known as celiac. This disease is triggered after consuming gluten. It is estimated that approximately 1% of the population is affected by celiac disease **Green and Cellier (2007)**. The only treatment is limiting the consumption of gluten. With this limitation, some nutritional deficiencies are manifested, such as low fibre content, absence of certain minerals, vitamins, proteins, etc. **Lee *et al.* (2009)**. With new food sources, ideas for fortifying gluten-free products are being developed.
Interest in insects has grown since 2013 when the FAO issued a statement on the benefits of eating them. Its production is believed to create a suitable replacement for conventional sources, and also contribute positively to reducing the impact on the environment **Oonincx *et al.* (2010)**, **Halloran *et al.* (2016)**. Eating insects is a common practice in Asian and Central American countries, where they are offered freely in markets. In Europe and the USA, their consumption remains low as for now **Liceaga (2021)**. **Govorushko (2019)** states that there are three reasons for accepting insects as a food source: human health, environmental factors, and socioeconomic benefits. There are several psychological barriers, however, that limit the development of entomophagy. These barriers primarily include neophobia, i.e. fear of consuming unknown food, or entomophobia **Hayati and Minaei (2015)**, and disgust. Some studies pay attention to the emotional perception of insects. One possibility that could increase consumers' interest in insects is to present them as something new or exotic **Wendin and Nyberg (2021)**. Informing about safety, origin, and production is also important to increase willingness to buy and consume insects **Haber *et al.* (2019)**. An alternative way to avoid the emotional fear of eating insects is to incorporate them into familiar products such as bread **Haber *et al.* (2019)**; **Montevecchi *et al.* (2021)**. Countless other products are also appearing on the market, including various types of sausages and pâtés. The development of these new products and technologies has mainly contributed to the modification of European legislation, thanks to which it is possible to produce insects as a new food. An opinion on the safety of insects was issued by the European Food Safety Authority (EFSA), which also proposed a list of insects that should be suitable for use in food and feed industry **Hardy *et al.* (2015)**. A number of experiments dealing with consumer acceptability of insects come from Belgium **Caparros Megido *et al.* (2016); Schouteten *et al.* (2016); Vanhonacker *et al.* (2013)**, Italy **Palmieri *et al.* (2019)**, Poland **Bartkowicz and Babicz-Zielińska (2020)** and other countries. The nutritional value and overall contribution of insects in food should be investigated in more detail. Due to population growth as well as food shortages in some parts of the world, an increased demand for additional protein sources is expected **Lange and Nakamura (2021)**.
The objective of our study is to evaluate the functional and sensory properties of gluten-free bars based on cricket powder.

MATERIAL AND METHODS

Gluten-free Production Process of Bars

For evaluation, gluten-free bars were prepared according to the recipe **Brisske *et al.* (2004)** with the content of house cricket (*Acheta domesticus*) powder (Catch-your-Bug, Germany). The recipe was modified to obtain a gluten-free product. Briefly, the loose ingredients (coconut flour, soy flour, soy protein isolate, whey protein concentrate, sugar, salt) were mixed and then liquid ingredients (fat, rice syrup, water) were added. The ratio of individual raw materials is shown in Table 1. Coconut flour was replaced by house cricket powder at a concentration of 0% (Ctrl), 5% (B5I), 10% (B10I), 15% (B15I), 20% (B20I), 25% (B25I). The bars were then baked in the oven at 180 °C for 10 min. Moisture was also measured using a moisture analyser (Ohaus, USA).

Table 1 Basic ingredients for the production of gluten-free bars

Ingredients	Content (%)
Vegetable shortening	18.2
Apple fibre	2.2
Rice syrup	14.5
Sugar	8.7
Coconut flour*	30.1
Soy flour	4.5
Soy protein isolates	2.5
Protein concentrate	14.5
Salt	0.4
Cane molasses	2.2
Water	2.2

* replaced by house cricket powder

Sample preparation

The bar samples were homogenized and prepared according to the methodology by **Zielińska *et al.* (2021)**. Briefly, 1 g of the sample was mixed with 10 ml of 75% ethanol and allowed to incubate for 120 min. This was followed by centrifugation at 3,000 rpm for 10 min in a centrifuge (Witeg Labortechnik GmbH, DE). The supernatant was used for further analyses.

Determination of Total Polyphenol Content

The total polyphenol content (TPC) was determined by means of the Folin-Ciocalteu reagent using gallic acid (MP Biomedicals, China) as a standard. 20 µl were taken from the sample to 96-well plate (Microtitration plate P No. V400917, Gamma group a.s., Czech Republic), 100 µl of Folin-Ciocalteu reagent and 80 µl of 7.5% sodium carbonate solution were added. After 30 minutes of incubation, the absorbance was measured at 765 nm (Tecan Austria GmbH, Austria).

Determination of Antioxidant Activity

The antioxidant activity was determined spectrophotometrically based on the colour change, the intensity of which corresponds to the concentration of antioxidants in the sample. The DPPH free radical and ABTS free radical were used for these assays. To determine the antioxidant activity, the supernatant from each sample was used and subsequently diluted 2 times. The chemicals used were from Sigma-Aldrich, USA.

ABTS

The working solution was created by mixing ABTS solution and potassium persulfate solution in a ratio of 1:1 using acetate buffer as a solvent according to the methodology by Xiao et al. (2020). The solution prepared in this way was left in the dark for 14 hours and then diluted again with acetate buffer until an absorbance of 0.74 ± 0.03 at 734 nm was reached. 200 µl ABTS working solution and 10 µl sample were pipetted into the 96-well plate. After 7 minutes of incubation in the dark, the absorbance was measured at 734 nm.

$$Scavening\ activity\ (\%) = \frac{Acontrol - Asample}{Acontrol} \times 100$$

The result was expressed as scavenging activity (%), where Acontrol corresponds to the absorbance of the ABTS solution and the Asample to the absorbance of the ABTS solution with the sample.

DPPH

The stable DPPH radical method was prepared as follows. 10 µl of the sample and 240 µl of DPPH solution prepared in 75% methanol was pipetted into a 96-well plate. After a 30-minute incubation in the dark at 37 °C, the absorbance at 517 nm was measured.

$$Scavening\ activity\ (\%) = \frac{Acontrol - Asample}{Acontrol} \times 100$$

The result was expressed as scavenging activity (%), where Acontrol corresponds to the absorbance of the DPPH solution and the Asample to the absorbance of the DPPH solution with the sample.

Sensory Analysis

On the second day after baking, the gluten-free bar samples were sensory evaluated by a panel of 16 briefly trained evaluators (10 women and 6 men). The average age of the panelists was 28 years. A total of 5 descriptors were evaluated by sensory analysis, using nine-digit categorical ordinal scales with described extreme values, where 9 corresponded to the highest pleasantness in the given descriptor. Using hedonic analysis, the descriptors of appearance, aroma, taste, texture, and overall pleasantness were evaluated. The samples were marked with a three-digit code. Still water was available as a neutralizer.

Statistical Analysis

The content of polyphenols and antioxidant activity were processed with statistical software of Xlstat 2022 (Addinsoft, France), the non-parametric Kruskal-Wallis test was applied. The Pearson correlation test was used to calculate the correlation coefficient. Data from the sensory analysis were processed with the statistical software of R version 3.3.3 (The R Foundation for Statistical Computing, Austria) using the principal component analysis method. All data were processed at a significance level of α = 0.05.

RESULTS AND DISCUSSION

The results of polyphenol content and antioxidant activity are summarized in Table 2. The highest amount of polyphenols expressed as gallic acid equivalent was determined in sample B25I (44.68 GAE/100 g), i.e. the sample with the highest content of insect powder. This sample also shows the highest antioxidant activity. Due to the identical composition, it can be assumed that the increase occurred due to the addition of house cricket powder. The Ctrl sample, which did not contain the insect component, shows the lowest TPC values and the lowest antioxidant activity values, determined by both, the DPPH and ABTS methods. A study by **Mudd *et al.* (2022)** confirms the antioxidant activity of banded cricket (*Gryllodes sigillatus*) proteins**. Di Mattia *et al.* (2019)** report that water-soluble extracts of the house cricket (*Acheta domesticus*) showed antioxidant activity, expressed as trolox equivalents, even 5 times higher than orange juice. Heat treatment also seems to be suitable for increasing antioxidant properties. In particular baking, which appears to be the most suitable, increases the antiradical activity of hydrolysates and peptide fractions **Zielińska *et al.* (2017)**. A study by **David-Birman *et al.* (2018)** confirms that the heat treatment of cricket powder improves the bioavailability its proteins and improves the function as an antioxidant. The addition of insects to gluten-free products causes an increase in antioxidant activity and at the same time brings enrichment with proteins of high nutritional value **Nissen *et al.* (2020)**.

Table 2 TPC and antioxidant activity of bars made with cricket powder

Gluten-free bar	TPC (mg GAE/ 100 g)	DPPH (%)	ABTS (%)
Ctrl	24.78 ± 0.63^{b}	4.10 ± 1.68^{c}	7.33 ± 1.02^{c}
B5I	27.17 ± 1.36^{ab}	8.27 ± 1.80^{bc}	15.09 ± 4.85^{c}
B10I	28.42 ± 0.58^{ab}	10.62 ± 1.17^{bc}	23.16 ± 2.78^{bc}
B15I	42.50 ± 3.38^{a}	25.23 ± 1.78^{ab}	39.92 ± 3.79^{ab}
B20I	36.44 ± 1.05^{ab}	23.62 ± 0.9^{ab}	39.73 ± 4.79^{ab}
B25I	44.68 ± 1.42^{a}	33.88 ± 2.30^{a}	50.17 ± 5.78^{a}

Key: Concentration of cricket powder 0% (Ctrl), 5% (B5I), 10% (B10I), 15% (B15I), 20% (B20I), 25% (B25I).

The bars were prepared and then baked all under the same conditions. The difference in moisture content was probably influenced by the addition of insect powder**. Zielińska *et al.* (2021)** describes the same trend in moisture content in a muffin-type bakery product. Muffins with the lowest addition of cricket powder showed the highest moisture (%).

Table 3 Moisture of bars made with cricket powder

Gluten-free bar	Moisture (%)
Ctrl	11.31 ± 0.51
B5	11.54 ± 1.36
B10	11.39 ± 0.65
B15	8.81 ± 0.18
B20	7.14 ± 0.91
B25	6.05 ± 1.21

Key: Concentration of cricket powder 0% (Ctrl), 5% (B5I), 10% (B10I), 15% (B15I), 20% (B20I), 25% (B25I).

Moisture content was positively correlated with added insect powder R=0.89 ($p < 0.05$). We explain this trend by the high ability of chitin to bind water, which leads to its gradual release and reduces the amount of free water in the product **Shahidi *et al.* (1999)**. The amount of water bound by chitin in food is influenced by its total content, but also by the pH of the food, because chitin is able to bind more water at a lower pH. The maximum ability is at pH 3 **Wang and Gunasekaran (2006)**. Chitin has similar properties to fibre and its presence in bars has a positive effect on reducing blood cholesterol, where one of the mechanisms is also the ability to bind in the small intestine, form complexes with bile and fatty acids **Muzzarelli (1996)**.

Products with the addition of 15-25% (B15-B25) house cricket powder would also meet the legislative requirement for water content required for durable bakery products produced in the Czech Republic due to the significant limitation of the growth of microorganisms thanks to the low availability of free water. The high content of antioxidants also stabilizes these products against fat oxidation, which also benefits their longer shelf life. It is obvious that the increasing concentration of insect meal reduces the amount of moisture in the product.

Sensory Analysis

The chart of the results of the principal components analysis, that was focused on individual gluten-free bar samples, explains 95.41% of the variability, with the first component explaining 82.19% and the second 13.22% of the variability. Based on the factor map of the variables (Figure 1), it can be said that the texture descriptor did not have a significant effect on the overall variability of the gluten-free bar samples. The closest correlation with overall acceptability was confirmed for the taste descriptor. Several statistically significant differences ($p < 0.05$) were found between the individual samples by hedonic analysis. These are presented in Tables 4 and 5, where statistically significant differences are highlighted using colour markings. In the case of Table 4, statistically significantly lower values ($p < 0.05$) are highlighted in red, statistically significantly higher values ($p < 0.05$) in the given descriptor are highlighted in grey.

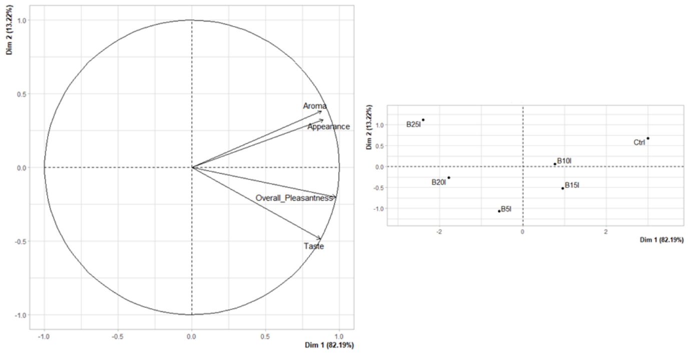

Figure 1 The results of PCA of sensory analysis of gluten-free bar samples: (**a**) Variables factor map. (**b**) Score plot for the mean points.

Table 4 Adjusted mean of hedonic evaluation of gluten-free bar samples with incorporated insect powder.

Gluten -free bar	Texture	Taste	Appearance	Aroma	Overall Pleasantness
Ctrl	6.13^{a}	7.25^{b}	7.19^{b}	7.31^{a}	7.44^{c}
B5I	5.75^{a}	6.94^{b}	6.63^{a}	6.38^{a}	6.75^{b}
B10I	6.44^{a}	6.88^{b}	6.69^{a}	7.06^{a}	7.00^{b}
B15I	6.06^{a}	7.13^{b}	6.69^{a}	6.94^{a}	7.13^{b}
B20I	6.00^{a}	6.38^{b}	6.44^{a}	6.50^{a}	6.31^{b}
B25I	5.88^{a}	5.69^{a}	6.56^{a}	6.56^{a}	6.00^{a}

Key: Key: Concentration of cricket powder 0% (Ctrl), 5% (B5I), 10% (B10I), 15% (B15I), 20% (B20I), 25% (B25I). No statistically significant differences in an individual descriptor were found between samples with the same index. Descriptor values that were evaluated as statistically significantly higher or lower are highlighted.

Table 5 Matrix with the p-values of the Hotelling's T2 tests for each pair of gluten-free bars.

	B10I	B15I	B20I	B25I	B5I	Ctrl
B10I	1	0.4962	0.1128	0.01617	0.1737	0.2202
B15I	0.4962	1	0.0811	0.009421	0.3717	0.183
B20I	0.1128	0.0811	1	0.264	0.2996	0.004635
B25I	0.01617	0.009421	0.264	1	0.03238	0.002745
B5I	0.1737	0.3717	0.2996	0.03238	1	0.003829
Ctrl	0.2202	0.183	0.004635	0.002745	0.003829	1

Key: Statistical differences between samples are highlighted in pink.

The control sample achieved the highest rating in all descriptors, however, there was a statistically significant difference ($p < 0.05$) only in the descriptors of appearance and overall pleasantness. On the contrary, the samples with the highest proportion of insect powder, namely B20I and B25I, achieved the lowest values in most descriptors. The statistically significantly lowest values ($p < 0.05$) were found for the B25I sample in the case of taste and overall pleasantness descriptors. Despite these differences, the evaluation of all samples containing different proportions of insect powder was positive – the mean values of all samples in all evaluated descriptors were always higher than 5 (neutral evaluation). These values ranged only in a narrow interval from 5,687 to 7,437, and thus the evaluation of the bar samples was relatively balanced. However, the degree of influence of insect powder addition on the sensory quality depends to a large extent on the specific food matrix, since in the case of pasta enriched with insect powder, the analysis showed that the addition of only 5% of insect powder did not lead to statistically significant changes in the sensory quality compared to the classic a whole grain variant of pasta **Duda *et al.* (2019)**. Similarly, in the case of a snack enriched with insect powder, the addition of up to 8% did not affect the sensory acceptability of the product **Cuj-Laines *et al.* (2018)**. **Talens *et al.* (2022)** analysed the effect of insect powder addition to a bakery product of a sponge cake type, where the addition of up to 7.5% of insect powder in combination with other alternative flours led to the achievement of an enriched product that was not statistically significantly different from the control sample. The matrix of gluten-free bars prepared by us seems to be particularly suitable for the purpose of fortification with insect powder, since the nutty flavour and brown colour introduced into the product by the addition of insect powder is actually desirable in the case of products of this type and is not perceived adversely **Talens *et al.* (2022)**.

CONCLUSION

This study showed that the evaluators perceived bars containing cricket powder rather positively. However, the sensory evaluation revealed that content higher than 25% has a negative effect on taste and overall pleasantness. Cricket powder in the product increased the amount of polyphenols and antioxidant capacity, which could be perceived as a value-added product for consumers. It was also shown that the content of cricket powder in foods of this type affected water content, what was proven by moisture measurements, where the increasing concentration of insect meal obviously reduced the amount of moisture in the product.

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