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Article

Wheat Bread Enriched with House Cricket Powder (Acheta domesticus L.) as an Alternative Protein Source

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Abstract: The house cricket (Acheta domesticus L.) is one of four edible insect species introduced to the EU market as a novel food and alternative protein source. Innovative products, such as cricket flour, are increasingly appearing on supermarket shelves and can offer an alternative to traditional cereals, while providing the body with many valuable nutrients of comparable quality to those found in meat and fish. The aim of this study was to investigate the possibility of using cricket powder as a substitute for wheat flour in the production of bread. The physicochemical properties of cricket powder were evaluated in comparison to wheat flour, such as water and fat binding capacity, pH, water activity, colour, water content, protein, fat, fibre content and amino acid and fatty acid profile. As a result of the echnological studies, the composition of breads with 5%, 10% and 15% replacement of wheat flour by cricket flour was designed and their quality characteristics (physicochemical, sensory and microbiological) were evaluated. Cricket powder was characterised by higher protein (63% vs. 13.5%) and fat (16.3% vs. 1.16%) content and a lower carbohydrate (9.8% vs. 66%) and fibre (7.8% vs. 9.5%) content as compared to wheat flour. The tested preparations had similar pH (6.9 and 6.8, respectively for cricket powder and flour), and fat-binding properties (0.14 vs. 0.27 g oil/g powder respectively for cricket powder and flour) but different water-binding properties and completely different color components. Despite the differences obtained in physicochemical properties for cricket powder and flour, most of the functional parameters obtained for bread with 5% cricket flour were not significantly different from the control sample. All breads had good microbiological quality after baking and during 7 days storage. The 10 and 15 per cent cricket flour treatments affected the darker colour of the breads and caused a significant increase in the hardness of the breads. In the overall sensory evaluation, the bread with 5% cricket flour did not differ significantly from the control bread, while the breads with higher levels of cricket flour had significantly lower overall scores compared to the control sample. In all cases, cricket flour increased the protein and fat content and decreased the carbohydrate content compared to the control sample. Considering nutritional properties such as high protein digestibility, breeding considerations, relatively low environmental impact and the possibility of intensive production in places where traditional agricultural production is not possible, insects seem to be the future of human nutrition.

Keywords: cricket powder; wheat flour; bread; sensory evaluation; fatty acids; amino acids

1. Introduction

The growing world population, depletion of freshwater resources, climate change, environmental pollution, increasing costs of livestock production are the most common factors contributing to the increased search for alternative food sources that are safe for humans and cheap to produce [1]. Among such alternative food sources, we can include edible insects, the consumption

2

of which is currently a widely discussed topic [2,3]. Insect consumption - entomophagy - is an integral part of the diet of at least 2 billion people in Asia, Latin America, Africa and Australia [4,5]. More than 2,000 species of edible insects are consumed worldwide [6]. The most commonly consumed species are those belonging to the orders Coleoptera (31.2%), Lepidoptera (17.1%), Hymenoptera (bees, wasps, ants, 15.2%), Orthoptera (grasshoppers, crickets, locusts, 13.2%) and Hemiptera (11.2%) [7]. Contrary to popular belief, insects are not only consumed in times of food scarcity. In many cultural circles (local culinary traditions), they are eaten by choice, both for their taste and their high nutritional value [7,8]. Insects provide high quality protein and nutrients comparable to meat and fish. The protein and fat content of insects varies from 25 to 75 per cent and 10 to 70 per cent in dry matter, respectively, and depends on the species and life stage, as well as environmental factors such as feeding, temperature and light [9]. Most insect species are rich in fatty acids. They are also rich in fibre and micronutrients such as copper, iron, magnesium, manganese, phosphorus, selenium and zinc, as well as vitamins such as riboflavin, pantothenic acid, biotin, and in some cases folic acid and carotenoids [7–12]. Considering both their nutritional properties, such as high protein digestibility, breeding considerations, relatively low environmental impact, and the possibility of intensive production in places where traditional agricultural production is not possible, insects appear to be the future of human nutrition [13–16].

As a result, the European Commission has taken steps to standardise edible insects from 2021. Currently, the insect species authorised as novel foods in the EU are *Tenebrio molitor* L., *Locusta migratoria* L., *Acheta domesticus* L. and *Alphitobius diaperinus* Panzer [17–21]. A further seven species are awaiting review. One of the edible insects that are farmed is the *A. domesticus* [22]. Its protein and lipid content is 20-25 and 4-7 g/100 g fresh weight, respectively, which is comparable to the content of these components in beef or chicken [23]. Its fat composition is 29-31% polyunsaturated fatty acids, and this insect is also a valuable source of vitamins [2]. The production efficiency of *A. domesticus* is 2.1, meaning that 2.1 kg of dry feed is required to produce 1 kg of food. In comparison, it takes 4.5 to produce 1 kg of edible product from poultry, 9.1 from pork and 25 kg of feed from beef [1]. House cricket is used for both food and feed purposes [24]. House cricket powder obtained from dried insects contains about 56% protein and about 27% fat with a predominance of C16:0 and C18:2 (cis-9,12) fatty acid fractions [23]. Food producers in the EU can now use whole individuals of A. domesticus in frozen, dried, powdered and partially defatted form in the production of many food products, including cereal bars, breads and rolls, crackers, dry pasta-based products, meat analogues, soups and soup concentrates, confectionery or processed meat products.

Currently, the main problem limiting the use of insects in the human diet in most European countries is the lack of acceptance and safety of this type of food. The main obstacle is undoubtedly cultural barriers and the associated lack of acceptance, reluctance or even fear among potential consumers. In contrast to the attitudes of populations in cultures where various insect species are considered traditional delicacies, consumers in European countries react with revulsion at the prospect of consuming organisms that are not commonly known as food, but as pests [25]. However, the introduction of insects into the diet does not necessarily mean consuming them in their natural form. There are many ways to process insects into a form that is more socially acceptable in developed countries, such as flour (powder), paste or extract (protein isolate. Studies by Tan et al. [25] and Hartmann and Siegrist [26] show that the way such products are prepared has a strong influence on their sensory acceptance by consumers who do not consume insects on a daily basis. According to de Oliveira et al. [27], a form reminiscent of conventional food, such as an accompaniment to minced meat or bread, was most accepted by consumers compared to eating insects whole.

In order to reduce insect-related food neophobia, this study investigated the possibility of obtaining breads that allow whole insects to hide, while maintaining a satisfactory level of sensory acceptance and increased nutritional value. Experimental breads were made with dough prepared from a mixture of wheat flour and house cricket powder at different levels.

2.1. Characteristics of cricket powder and whole wheat flour

The chemical composition of whole wheat flour and cricket protein powder used for bread making is presented in Table 1. The protein content in cricket powder was about 63 g/100 g, it was several times higher than in wheat flour (13.5 g/100 g). Cricket powder was also characterized by higher fat content as compared with wheat flour (16,29 vs. 1.16 g/100 g). However, wheat flour had higher fibre content (9.50 vs. 7.80 g/100 g) and much higher carbohydrates content (66.04 vs. 9.83 g/100 g) than cricket protein. Similar results on the chemical composition of cricket powder were presented by Rumpold and Schüter, Lucas-González et al. and Mafu et al. [2,28,29]. As confirmed in a study by Kowalski et al. (2022) [30] edible insects are considered to be a food source with high nutritional value due to their high content of proteins, fats, vitamins and minerals.

Table 1. Chemical composition of cricket protein powder and whole wheat flour.

Nutrients content	Cricket protein powde	erWhole wheat flour
Fat [g/100 g]	16.29±3.10	1.16 ±0.23
Protein [g/100 g]	63.00±7.56	13.5 ±1.62
Carbohydrates [g/100 g]	9.83±0.95	66.04 ± 1.43
Fibre [g/100 g]	7.80±1.20	9.5 ±1.5

The tested flours differed statistically significantly (p<0.05) in terms of water holding capacity and water activity and colour parameters, while they were similar in terms of pH and fat absorption (Table 2). Wheat flour had lower water absorption properties of about 1.52 g water/g flour compared to a mean value of about 2.53 g water/g obtained for cricket powder. The differences obtained in water absorption properties may be due to the higher protein content of cricket powder 63 g/100 g compared to wheat flour - 13.5 g/100 g.The water activity parameter ranged from 0,18 for cricket powder to 0,43 for wheat flour. According to Tiwari and Jha [31], a low aw value in the range of 0.1 to 0.33 is favorable for the microbiological stability of products, which facilitates storage and distribution. The L* colour parameter values of cricket powder and wheat flour were 35.14 and 81.58, respectively. Wheat flour had a much lighter colour, which was easily visible to the naked eye (Figure 1). The other colour parameters, a* and b*, also showed significant variations (p<0.05). The colour parameter a* values obtained for cricket powder and wheat flour were 4.56 and 4.74 respectively, and the parameter b* values for cricket powder and wheat flour were 25.22 and 21.58 respectively. The results show that wheat flour was characterised by a slight colour shift towards a red hue compared to cricket powder, which in turn showed a greater colour shift towards a yellow hue. In addition, as can be seen in Figure 1, wheat flour was characterised by a high particle size in contrast to cricket powder, where chitin elements were very prominent.

A similar result was reported by other researchers, including Mafu et al. [29], who stated that cricket powder showed a low value in lightness L* (40.81 ± 0.61) and redness a* (4.9 ± 0.53) and a high value in yellowness b* (15.13 ± 2.02) . In another study, Lucas-González et al. [28] reported that cricket powder showed a low value of L* (41.62 ± 0.61) , caused by the brown colour of the outer chitin skeleton, which is characteristic of insects.

Table 2. Functional properties of cricket protein powder and whole wheat flour.

Physicochemical properties	Cricket powder	Whole wheat flour
pН	6.90±0.02	6.77±0.13
Fat absorption capacity [g oil/g powder]	0.14±0.12	0.27±0.05
Water holding capacity [g water/g powder]	2.52±0.54 b	1.52±0.14 a
$a_{\rm w}$	0.18±0.00 a	0.43±0.00 b
L*	35.14±0.53 a	81.58±1.34 b
a*	4.56±0.10 a	4.74±0.48 b

Values followed by different small letters (a, b) in row are significantly different (p \leq 0.05). Values are means of three replicates \pm standard deviation.

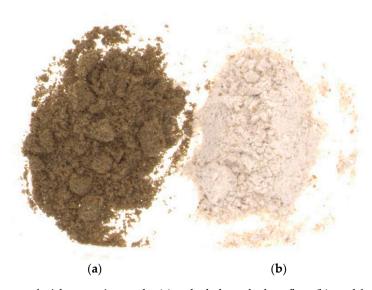


Figure 1. Pictures of cricket protein powder (a) and wholemeal wheat flour (b) used for bread making.

Significant differences were observed in the fatty acid profile of cricket powder and whole wheat flour (Table 3). The protein powder obtained from cricket was characterised by a higher content of saturated fatty acids (about 35% of fat content) and monounsaturated fatty acids (about 26% of fat content), and a much lower level of polyunsaturated fatty acids (about 34% of fat content) in comparison with the wheat flour sample (the content of the above-mentioned fatty acid groups was about 16%, 14%, 64% of fat content, respectively). In addition, wholemeal wheat flour showed a more than threefold higher content of fatty acids from the n-3 family (about 3.6% of fat content vs. 1.0% of fat content) and almost twice the content of acids from the n-6 family (about 60.5% of fat content vs. 32.1% of fat content). The fatty acid profile of cricket powder is similar to that of pork [32].

Table 3. Fatty acid profile of cricket protein powder and whole wheat flour .

Fatty acid profile	Cricket protein powde	rWhole wheat flour			
Saturated fatty acids [% of fat content]					
(C14:0) myristic acid	0.50±0.20*	0.06 ± 0.03			
(C15:0) pentadecanoic acid	0.08 ± 0.04	0.06 ± 0.03			
(C16:0) palmitic acid	23.98±4.80	14.80 ± 2.96			
(C17:0) margaric acid	0.15 ± 0.06	0.07 ± 0.03			
(C18:0) stearic acid	9.66±1.94	0.97 ± 0.30			
(C20:0) arachidic acid	0.29 ± 0.12	0.12 ± 0.05			
(C22:0) behenic acid	< 0.05	0.17 ± 0.07			
(C24:0) lignoceric acid	< 0.05	0.14 ± 0.06			
Monounsaturated fatty acids [% of fat content]					
(C16:1w7) palmitoleic acid	0.67±0.21	0.12 ±0.05			
(C18:1w9) oleic acid	24.64±4.93	12.99 ± 2.60			
(C18:1w7) cis-11-vaccenic acid	0.60 ± 0.18	1.03 ±0.21			
(C18:1w9t) trans elaidic acid	0.12 ± 0.05	< 0.05			
Poliunsaturated fatty acids [% of fat content]					
(C18:2w6) linoleic acid (LA)	32.10±6.42	60.53 ±12.11			
(C18:2 ct) cis-9, trans-12 octadecadienoic acid	0.58 ± 0.18	< 0.05			

(C18:2w6t) trans linolelaidic acid	0.13±0.06	< 0.05			
(C18:2 tc) trans-9, cis-12 octadecadienoic acid	0.68±0.21	< 0.05			
(C18:3w3) cis-9, 12,15 alpha-linolenic acid (ALA)	0.99 ± 0.30	3.64 ± 0.73			
(C20:2) cis-11,14- eicosadienoic acid	0.43±0.18	0.08 ± 0.04			
Contribution of individual fatty acid groups [% of fat content]					
Saturated fatty acids	34.66±6.94	16.39 ±3.28			
Monounsaturated fatty acids	25.91±5.19	14.30 ±2.86			
Polyunsaturated fatty acids	33.52±6.71	64.25 ±12.85			
Trans fatty acids	1.51±0.31	<0.55 ±0.17			
Omega 3 fatty acids (ALA, EPA, DHA, ETE, DPA)**	0.99±0.30	3.64 ± 0.73			
Omega 6 fatty acids (LA, GLA, ARA, DGLA)***	32.10±6.42	60.53 ±12.11			

^{*)} result obtained \pm extended measurement uncertainty (relative) with p<0.05. **) EPA - (C20:5w3) cis-5,8,11,14,17- eicosapentaenoic acid; DHA - (C22:6w3) cis-4,7,10,13,16,19- docosahexaenoic acid; ETE - (C20:3w3) cis-11,14,17- eicosatrienoic acid; DPA - (C22:5w3) cis-7,10,13,16,19- docosapentaenoic acid; ***) GLA - (C18:3w6) gamma-linolenic acid; ARA - (C20:4w6) arachidonic acid; DGLA - (C20:3w6) cis-8,11,14- eicosatrienoic acid.

Due to the approximately five times higher protein content in the cricket powder (about 63%) compared to the content of this macronutrient in the wholemeal wheat flour (about 13%), the content of all amino acids tested was also higher in the cricket powder. The amino acid content of the cricket powder was between two (for glutamic acid) and twelve (for alanine) times higher than their content in the wheat flour (Table 4).

Table 4. Aminoacids p	orofile of cricket	protein powder and	l whole wheat flour .
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Aminoacids profile	Cricket protein powder	Whole wheat flour
Aspartic acid	5.31±0.02*	0.64±0.02
Threonine	2.37±0.02	0.36 ± 0.02
Serine	2.69±0.02	0.63±0.02
Glutamic acid	6.98±0.02	3.91±0.02
Proline	3.66±0.02	1.31±0.02
Glycine	3.01±0.02	0.51±0.02
Alanine	5.32±0.02	0.45 ± 0.02
Valine	3.57±0.02	0.54 ± 0.02
Methionine**	1.08±0.02	0.19±0.02
Isoleucine	2.43±0.02	0.43 ± 0.02
Leucine	4.69±0.02	0.89 ± 0.02
Tyrosine	3.66±0.02	0.37±0.02
Phenylalanine	2.38±0.02	0.61±0.02
Lysine	3.57±0.02	0.34 ± 0.02
Histidine	1.41±0.02	0.30±0.02
Arginine	3.90±0.02	0.63±0.02
Taurine	0.22±0.02	< 0.02
Hydroxyproline	0.04 ± 0.02	< 0.02
Cyst(e)ine, calc. from cysteic acid	0.65±0.02	0.29±0.02

^{*)} result obtained \pm extended measurement uncertainty (relative) with p = 95 %, k = 2; **) calc. from methionine sulfone.

2.2. Assessment of quality parameters of designed cricket bread

2.2.1. Physicochemical properties

Table 5 shows the physical parameters, i.e. the colour components, specific gravity, water activity and hardness of the breads with 5, 10 and 15 per cent addition of cricket powder and the

control bread. Statistically significant (p<0.05) differences were obtained for most of the parameters tested, except density.

Colour is one of the most important characteristics of bakery products and, along with texture, can influence consumer acceptance. The values of the colour component (L*) ranged from about 40 for bread with 10 and 15% cricket powder addition to about 44-45 for the control sample and with 5% cricket powder addition. The results obtained indicate that the addition of cricket powder significantly (p<0.05) affects the darker colour of the bread crumb, however, the lowest level of addition (5%) does not cause significant changes in the brightness of the bread. The values of the colour parameters a* and b* were significantly (p<0.05) highest in the control sample, meaning it had the most intense red and yellow colour compared to the samples with added cricket powder. Similar results were obtained in studies by Gantner et al. [33] and Khuenpet et al. [34] on the effect of adding mealworm powder on the colour of bread and muffins. In these authors' study, the addition of mealworm powder contributed to a significant (p<0.05) reduction in the red and yellow colour of the tested bakery products compared to the control. These results are also in line with those of other authors, where the addition of cricket flour reduced dough redness and yellowing Bartkiene et al. [35]. The colour changes in a* and b* of the bread crumb may be due to the natural colour of the whole wheat flour, while the differences in L* brightness are due to the darker brown colour of the cricket chitin. González et al. [36] reported that the color of bakery products directly depends on the color of raw material.

In this paper, the total colour difference (ΔE) and the browning index (BI) were calculated from the L*a*b* colour parameters studied. The results obtained show an increase in the value of ΔE as the proportion of insect powder in the bread composition increases. Based on the study of Bellary [37], it is known that the colour difference ΔE >3.0 is visually noticeable to inexperienced consumers. In the present study, the values of the total bread colour difference (ΔE) ranged from 1.03 for the bread with the lowest addition of cricket powder to 6.27 for the sample with 10% addition. These results indicate that the breads developed with 10 and 15% cricket powder addition have significant colour differences compared to the control sample, which may result in lower consumer acceptability compared to traditional wheat breads. No effect of cricket powder addition on the BI value was observed, which may mean that the contribution of the component with higher protein content (cricket powder vs. bread flour) did not contribute to the intensity of the Maillard reaction that occurs during the baking process.

The hardness parameter showed that the textural values of the bread were significantly influenced by the addition of 10 and 15% cricket powder. The differences obtained may have been due to the different content of individual nutrients (fat, fibre, gluten protein) in the samples tested, which shaped the texture of the bread in different ways. The lowest addition of 5% insect powder did not significantly (p > 0.05) affect hardness compared to the control bread. These results partially confirmed the study by Kowalski et al. [30], where a decrease in bread hardness was observed at 10% addition of cricket flour, and a significant increase in the parameter studied at 20 and 30%. Similar results were presented by Khuenpet et al. [34]. According to de Oliveira et al. [27] and González et al. [36], high hardness of the bread indicated the resistance to deformation and long time needed to chew bread product before swallowing. On the other hand, García-Segovia et al. [38] and Gonzalez et al. [36] reported no significant differences in the hardness of breads where wheat flour was replaced by insect flour.

No significant differences in terms of water activity were observed among the analyzed samples and was 0.95–0.96. Different results were obtained by Ruszkowska et al. [39], where snacks with 2 and 4% cricket powder added had lower water activity compared to the control sample, which directly affected their crispness. In a study by Mafu et al. [29] bread with 10% and 25% cricket powder had the highest moisture content and was significantly different (p < 0.05) from the control bread. According to the authors, who did not show a linear effect of insect protein addition on bread moisture content, the dough mixed with gluten protein and cricket protein particles is gradually, but inconsistently moistened as without cricket powder. This may account for the variability in moisture

content of the fortified bread, which should be investigated in the future, especially since this parameter is closely related to the hardening process of starch products [39].

Table 5. Physicochemical properties of obtained breads loaves.

Physicochemical properties	s.C	CR5	CR10	CR15
L*	45.07±0.22	a44.24±0.68	a40.42±0.68	b40.65±0.56 b
a*	6.20±0.07 °	5.78±0.12 b	5.11±0.06 a	5.88±0.12 b
b*	27.89±0.01	c27.11±0.09	b25.11±0.52	a26.78±0.10 b
ΔE	-	1.03	6.27	4.15
Density [g/cm ³]	13.91±0.18	b13.21±0.42	a13.90±0.18	a12.91±0.29 b
$a_{ m w}$	0.96±0.05 a	0.95 ± 0.03^{a}	0.96 ± 0.00^{a}	0.96 ± 0.00^{a}
Hardness [N]	30.08±4.17	a 31.93±4.99a	55.89±5.31 ^t	39.94±4.41°

Values followed by different small letters (a-c) in row are significantly different ($p \le 0.05$). Values are means of three replicates \pm standard deviation.

2.2.2. Nutritional value

Replacing part of the flour with a cricket protein preparation resulted in obtaining breads with higher protein and fat content as compared to control sample (Table 6). Breads with 10 and 15% cricket protein addition showed more than 20% of energy value obtained from protein, thus meeting the requirements for products with high protein content, according to the Regulation EU No. 1924/2006 of the European Parliament and of the Council of December 20, 2006 on nutrition and health claims [40]. Higher protein content also results in higher levels of amino acids, which was confirmed in the studies of Mafu et al [29] and Wieczorek et al [41], who observed a significant increase in the content of essential amino acids after baking breads with cricket powder addition. With an increase in the addition of cricket protein preparation to the breads, a decrease in carbohydrate content and a slight decrease in dietary fiber were observed compared to the control bread. Nevertheless, the decrease in fiber content was not so significant that the developed breads could not be labeled with the nutrition claim: source of fiber in accordance with the regulation stated above [40].

Table 6. Energy value and nutrients content in 100 g of designed breads

Nutritional value	C	CR5	CR10	CR15	
Energy value (kJ/kcal)	877/21	1890/21	3904/21	6916/219	
Fat (g)	0.7	1.1	1.6	2.0	
Carbohydrates (g)	39.8	38.1	36.5	34.8	
Fibre (g)	5.8	5.7	5.7	5.6	
Protein (g)	8.2	9.7	11.2	12.6	
% energy from protein15.7 18.2 20.7 23.0					

2.2.3. Sensory evaluation

The results of sensory evaluation revealed that the addition of cricket powder influenced assessed visually color and porosity of the samples (Figures 2 and 3). The intensity of bread crumb color increased and porosity decreased with the higher cricket powder addition. The crumb of CR10 and CR15 samples was significantly darker and less porous than the control sample. The color is an important bread feature influencing consumer choice and acceptance. Significant effect of insect powder addition on the color of bread and other bakery products was also demonstrated in other authors studies [30,33,36]. The influence on crumb porosity may be due to the fact that cricket powder does not contain gluten [42]. Moreover, cricket powder is made from adult insects, having an exoskeleton, head, eyes, lower jaw, antennae, legs and wings. As a consequence, it contains some amounts of chitin and is also characterized by a coarse-grained structure, which may affect the baking properties of the flour [43].

8

Visually perceived differences in the porosity of the crumb did not affect the consistency attributes assessed in the oral cavity. All bread samples were described as quite soft, moderately adhesive, and moist, regardless of the cricket powder addition level. These results are in line with González et al. [36] studies which showed that that replacing wheat flour with cricket powder at the level of 5% didn't significantly affect bread texture parameters measured instrumentally. Nevertheless, replacing wheat flour with cricket powder at levels of 10 and 15% contributed to an increase in crumb hardness in instrumental evaluation, but this did not result in an increase in oral feeling as assessed by experts in sensory tests.

With regard to odor attributes, bread enrichment with the cricket powder influenced the perceptibility of the bread odor, the intensity of which was significantly higher in the control sample compared to CR10 and CR15. The intensity of the sweet and nutty odor remained at a similar level, independent of the addition of cricket powder. Recipe modification influenced also the intensity of bread flavor and bitter taste. The CR15 sample, compared to the control sample, was characterized by a significantly lower intensity of the bread flavor and a higher intensity of the bitter taste. The intensity of the sweet and nutty flavors were similar in all bread sample. In breads with a higher level of cricket powder addition (CR10 and CR15), the presence of an off-flavor unusual for bread was found. As indicated in the literature, cricket powder is characterized by a strong flavor described as a crustacean-like, cooked legumes-like and earthy [43]. Therefore, too high a level of cricket powder addition to bread negatively affects the sensory quality.

The differences in the intensity of sensory attributes affected the assessment of the overall bread sensory quality, defined as an appropriate harmonization of sensory features. The highest sensory quality, not significantly different from the control sample, was characterized by the CR5 sample. The sensory quality of the CR10 and CR15 samples was statistically significantly lower than the control sample. Bakery products enrichment with powder forms of edible insects is challenging, as fortification impacts the sensory properties of the final product with a consequent reduction of palatability and overall consumer acceptability. Thus, including insect flours without negatively altering sensory quality is only possible to a certain level. Osimani et al. [44] found that the addition of cricket powder at the level 10%, 20% and 30%, markedly affected the acceptance of the bread due to the notable flavor and taste of the insect-based ingredient. The highest degree of palatability was found for the control bread, while the samples of bread with the addition of cricket powder at the level of 20 and 30% were not accepted by consumers.

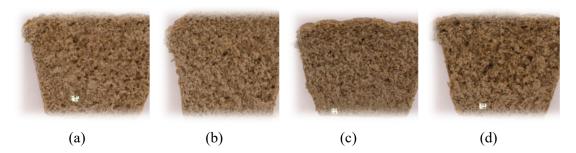


Figure 2. Pictures of prepared bread. (a) - control sample; (b) - C5; (c) - C10; (d) - C15.

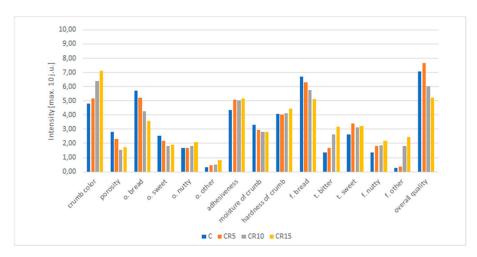


Figure 3. The sensory characteristic of the breads with addition of cricket powder (f. – flavor, t. – taste, o. – odor).

2.2.4. Microbiological evaluation

The results of microbiological analysis of tested breads during 7 days of storage are presented in Table 7. Total viable counts (TVC) after baking in all of the examined breads were low (from 3.71 log CFU g-1 for control sample to 4.52 log CFU g-1 for CR10 sample). There were no statistically significant differences (p>0.05) in TVC between the breads with different amounts of insect powder. After 2 days after baking, there was an increase in TVC in all variants of the breads of about 2-3 logarithmic orders. High level of Total viable counts (from 7. 37 log CFU g-1 for CR5 to 9.44 log CFU g-1 for CR15 sample, respectively) was found in control, and breads with insect powder at 7 day of storage. Malomo et al., [45] also observed similar increase of Total viable counts to approx. 8-9 CFU g-1 in the breads with 0, 1, 3% of cottage cheese (warancashi), stored 9 days under ambient temperature.

Yeasts and molds are the main microorganisms responsible for the deterioration of bread [46]. Water activity of bread (0.95-0.96), moisture content (about 40%), pH (5-6), and present of nutrients favor fungal development [47–54]. Fungal spoilage affects the safety aspects due to mycotoxigenic fungi, limited shelf life of bread, contributes to economic losses, and household bakery products waste, and decreased sensory quality [47,55].

There was no presence of yeasts and molds after baking in the breads with, and without the addition of insect powder. The high temperature during bread manufacturing 'process eliminates natural microbiota from raw material [56]. Bread recontamination with yeast and molds is possible after baking at the packing, cooling, and storage stage [57,58]. Special attention must be paid to hygienic quality of air due to fungal particles dispersed [58]. After 7 days of storage all tested breads characterized by non-statistically significant, and relatively low counts of yeast and molds (approx. 4 log CFU g-1).

Table 7. Microbiological analysis of tested breads after production and after 2 and 7 days of storage.

Samples	Days	TVC [log CFU g-1]	Y&M[log CFU g-1]
	0	3.71±0.28 a	nd
C	2	7.10±0.01 bd	3.67±0.02a
	7	8.39±0.31 ^c	4.33±0.07 b
	0	4.30±0.06 d	nd
CR5	2	6.49±0.39 de	nd
	7	7.37±0.34 ь	4.34±0.08 b
	0	4.52±0.16 ^d	nd
CR10	2	7.19±0.20 b	3.43±0.02 a
	7	8.53±0.20 ^c	4.40±0.11 b

	0	4.32±0.12 d	nd	
CR15	2	7.17±0.19 b	nd	
	7	9.44±0.29 df	4.29±0.08 b	

The values are expressed as means \pm SD; means in the same column followed by different lowercase letters (a-f) are significantly different (p<0.05); TVC - Total Viable Counts; Y&M -Yeast and Molds; nd – not detected.

3. Materials and Methods

3.1. Materials

The tested materials were cricket powder, wholemeal wheat flour and breads, where wholemeal wheat flour was replaced with cricket powder at levels of 5, 10 and 15%. For the preparation of wheat bread (control sample) and wheat bread with insect flour, the following materials were used: commercial whole wheat bread flour type 1850 (Melvit, Poland), salt, dried yeast purchased on the local market and cricket (*A. domesticus*) protein powder produced by SENS Food LTD (London, United Kingdom).

3.2. Methods

3.2.1. Functional properties of cricket powder and whole wheat flour

3.2.1.1. The pH measurement

The pH was measured using the potentiometric method with a portable pH meter (model 205, Testo AG, Germany). The pH values were measured in a 5% solution of wheat flour and cricket powder in water. Values were measured in tripllicate.

3.2.1.2. The water activity (aw) measurement

To measure a_w, 5g of whole wheat flour and cricket powder were placed in a measuring cup in the device (AquaLab 4TEV, Munich, Germany) and the measurement was made in triplicate.

3.2.1.3. The water holding capacity (WHC) measurement

WHC analysis was performed on 1 g of cricket protein powder or whole wheat flour. The sample was homogenised using an ultrasonic homogeniser (Hielscher UP400ST, Berlin, Germany) with 30 cm3 of distilled water and then centrifuged for 15 min (6000 RPM, 0 °C) in a centrifuge (MPW-380 R, MPW Med. Instruments, Warsaw, Poland). After flooding with complementary water, samples with sediment were left upside down for 10 min and then weighed. The WHC was determined in triplicate for each sample by weighing the samples at the beginning and after centrifugation calculating the difference in mass. The measurement was expressed as grams of water absorbed per gram of flour or powder.

3.2.1.4. The fat absorption capacity (FAC) measurement

FAC was determined using a similar procedure but with rapeseed oil. The FAC was expressed as the amount of oil per gram of flour or powder and was determined in triplicate.

3.2.1.5. The Colour measurement

The IRIS artificial eye visual analyser (AlphaMos, Tuluse, France) was used to measure wholemeal wheat flour, cricket powder and bread colour. Colour measurements were carried out triplicate. The loaf was randomly selected from the batch. The colour components were read in the same way as for the flour measurements. Colour was recorded on the CIE L*a*b* scale in terms of brightness (L*) and colour (a*-redness; *-yellowness). In addition the total colour difference (ΔE) between the reference sample and the test sample was calculated using the following equation: ΔE = $\sqrt{\Delta L^2 + \Delta a^2 + \Delta b^{*2}}$. The browning index was also calculated using following eq.:

3.2.1.6. Chemical composition

Chemical tests on cricket powder and wholemeal wheat flour assessed water, protein, fat, dietary fibre and amino acid and fatty acid profiles. These tests were carried out in an external, accredited analytical laboratory according to the test procedures used there.

3.2.2. Development of the composition of bread with the addition of cricket preparation

The bread was made in three variants with different amounts of cricket protein powder, added to partially replace whole wheat flour at 5, 10 and 15%. The control bread was made without the addition of insect powder. Water, dried yeast, salt, and sugar were placed in a mixing bowl and mixed for 3 minutes at 37 °C. Then flour was added, and the dough was mixed for 3 minutes. The dough was then left to rise in a warm place for 1 hour. Table 8 shows the complex combination of the samples tested.

Table 8. Recipes [%] for bread made with whole wheat flour (C), whole wheat flour with the addition of 5% (CR5), 10% (CR10) and 15% (CR15) cricket powder.

Samples	Wholemeal wheat	flourCricket powder	Dried yeasts	Sugar	Salt	Water
С	58.7	0.0	0.8	0.7	0.7	39.2
CR5	55.8	2.9	0.8	0.7	0.7	39.2
CR10	52.9	5.9	0.8	0.7	0.7	39.2
CR15	49.9	8.8	0.8	0.7	0.7	39.2

3.2.3. Physical parameters of designed breads

3.2.3.1. The density measurement of prepared breads

The density of bread was determined by pouring it into a measuring cup filled with seeds. A slice of bread was placed in the centre. By observing the increase in the volume of the mixture and knowing the mass of the sample, the density of the samples tested was determined. The measurement was carried out in three repetitions, for each bread sample. The density of bread was calculated following equation:

Density
$$\left[\frac{g}{cm3}\right] = \frac{\text{loaf weight [g]}}{\text{loaf volume [cm3]}}$$

3.2.3.2. The hardness measurement of prepared breads

The mechanical properties of the crumb, expressed as hardness, were measured on the discs of thickness (20 m) using a texture analyser (Stable Micro Systems TA. XT2i, USA). The settings used were: 25 mm diameter cylindrical aluminium probe, 50% deformation, 5 s pause between measurements and 5 mm/s probe movement speed. Measurements were made in six replicates at room temperature. Three slices of each loaf (from the centre) were measured.

3.2.4. The nutritional value assessment of prepared breads

The nutritional value, i.e. protein, fat, fibre, carbohydrate content, of the designed breads was calculated on the basis of the chemical composition of the components both after analytical determinations (cricket powder and wheat flour) and on the basis of the nutritional value tables (other components) [59].

3.2.5. The microbiological evaluation of prepared breads

The evaluation was performed using the plate method. Total viable counts (TVC) was determined according to ISO 4833 1:2013-12/A1:2022-06 [60] on nutrient agar (Merk, Germany). Plates were incubated at 30C for 72h. The total number of yeast and molds (Y&M) were analyzed according to ISO 21527-1:2008 [61] on YGC agar (Sabouraud Dextrose with Chloramphenicol LAB-Agar, Biomaxima, Poland). Plates were incubated at 25oC for 72-120 h. The results of the viable counts were expressed as mean of log CFUg-1 ± standard deviation.

3.2.6. Sensory evaluation

For sensory assessment of bread samples, the scaling method according to ISO 4121:2003 [62] was used. The evaluation was performed by a trained panel (n=6) fulfilling the requirements of ISO standard 8586:2012 [63]. The assessors evaluated the intensity of attributes in two sessions using a 10 cm linear unstructured scale ranging from "none" to "very strong" (for odour, flavor and taste descriptors) and "low" to "high" for texture attributes evaluated in the oral cavity (adhesiveness, moisture of crumb and hardness of crumb) as well as the overall quality of the samples. For visual attributes, crumb color was assessed on a scale ranging from "pale" to "dark", whereas crumb porosity was evaluated on a scale anchored from "low porosity" to "high porosity". Since the evaluation of bread samples was performed in two independent sessions (repetitions), the average values of each sensory attribute was based on twelve individual results. Individual bread samples of the same size were presented in transparent plastic containers (200 mL) coded with 3-digit numbers and covered with lids. Still mineral water was used as a taste neutralizer. The order of sample presentation was balanced to account for first order and carry-over effects. Assessment was performed in the sensory laboratory equipped with individual booths with white light. The laboratory met general requirements of ISO standard 8589:2010 [64].

3.2.7. Statistical analysis

Statistica 13.0 software (Tibco Software Inc., Palo Alto, CA, USA) was used for all statistical processing. Analysis of variance (ANOVA) was used for dependent groups with post hoc analysis of Duncan's test at a significance level of p < 0.05.

4. Conclusions

The results of the present study indicate that cricket protein powder can be used for the production and protein enrichment of wheat bread. The cricket flour addition variants used in all cases increased the protein, fat and fibre content and decreased the carbohydrate content of the product. The fortification of wheat bread with cricket flour at 10 and 15 per cent affected the colour components and caused a significant increase in hardness measured instrumentally, which did not lead to an increase mouthfeel as assessed in sensory evaluation. For most of the physico-chemical parameters and sensory evaluation, the breads with 5% cricket flour did not show any significant differences compared to the control sample. All breads had a good microbiological quality after baking and during 7 days of storage. Considering the results obtained and the fact that insects provide a sufficient supply of energy and protein in the human diet, are a source of fibre, vitamins and micronutrients and have a high content of mono- and polyunsaturated fatty acids, the suitability of cricket powder for protein enrichment of bakery products is confirmed. Future research should focus on the potential safety problems of edible insects (allergic reactions and contamination with pathogenic micro-organisms) and the development of appropriate recipes, in order to create a positive consumer attitude towards innovative insect-based foods.

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