HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

EVALUATION OF THE SENSORY, NUTRITIONAL, AND CERTAIN TECHNO-FUNCTIONAL PROPERTIES OF INSECT-ENRICHED FOODS

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1. INTRODUCTION AND OBJECTIVES

The term *entomophagy* is of Greek origin, a combination of the words *entomon* and *phagein*, meaning insect and to eat. Entomophagy is not a recent phenomenon, it dates back to prehistoric times and is still part of the gastronomy of 140 countries, such as Japan, Mexico and Nigeria. There are more than 2,000 species categorised as edible, including beetles, ants, wasps and caterpillars, in almost all stages of development. The most consumed species are from the orders Coleoptera, Lepidoptera, Hymenoptera and Orthoptera. As identified by the European Food Safety Authority (EFSA) in 2015, the most promising edible insect species include *Acheta domesticus*, *Locusta migratoria*, *Bombyx mori*, *Alphitobius diaperinus* larvae, *Tenebrio molitor* larvae and *Hermetia illucens* larvae, which are already playing an increasingly important role in feed production.

Insects have not been a food source for the human population of Europe and North America, but rapid population growth, depletion of the planet's resources and the negative effects of climate change mean that alternative sources of protein to traditional ones may be needed to meet nutritional needs. Such sustainable alternative protein sources could be edible insects; therefore, their potential uses, nutritional value, food and feed safety risks, environmental impact and consumer acceptance have become one of the priorities in the field of food and nutrition science.

Edible insects are promising from a nutritional point of view. Their energy value and composition are significantly influenced by species, sex, developmental stage, the insects’ lifestyle, living conditions and environment, the quality and composition of the feed used and the processing method. Literature data indicate that their protein content is high (35-60% on a dry matter basis), but may be overestimated by protein determination methods based on nitrogen determination due to their exoskeleton’s chitin content. The quality of insect proteins and their amino acid composition are promising, similar to those of traditional animal protein sources and soybeans, but the determination of these compounds is difficult due to the high variability of their composition. The bioavailability of insect protein is also difficult to determine due to their chitin content, therefore more data on their protein content are still needed.

Their fat content is highly variable (10-60% on a dry matter basis), with larvae and pupae having a significantly higher fat content than adults. Their lipid profile is mainly dominated by triglycerides and phospholipids, but they also contain unsaturated fatty acids such as oleic, linoleic and linolenic acids. Their carbohydrate content, generally expressed in the literature as the nitrogen-free extract content, is not significant.

Insects also have a promising micronutrient content: they are good sources of riboflavin, pantothenic acid and biotin, and have significant levels of phosphorus, magnesium, iron, calcium, potassium and zinc, but further research is needed to map those micronutrients' bioavailability. The main source of dietary fibre in insects is chitin (10-20 g/100 g), which builds their exoskeleton. This polysaccharide of *N-acetylglucosamine* units is considered to be a water-insoluble fibre, but some research suggests that the enzyme chitinase, may be present in an active form in the human gastrointestinal tract when insects are consumed regularly. Although insects' chitin content can be reduced to improve the digestibility of insect proteins, it is important to note that chitin as a dietary fibre can help maintain the health of the cardiovascular and gastrointestinal systems.

Based on the available literature, edible insects may also contain antinutritional factors that may inhibit the absorption and bioavailability of macro- and micronutrients, but these compounds can be eliminated by using appropriate processing technologies.

In 2015, the European Food Safety Authority (EFSA) published a comprehensive study on food and feed safety issues related to edible insects, which concluded that insects pose similar risks (microbiological, chemical, physical hazards) to those known for conventional livestock production, but further data are needed to fully map these risks. However, it is important to underline that insects often cause allergic sensitisation through the respiratory tract and skin, which can lead to severe, life-threatening symptoms. When assessing the risk of allergenicity of edible insects, data from other arthropods such as arachnids, crustaceans (dust mites, lobsters, crabs) are used, as those who are allergic to them may be cross-allergic. Researchers have identified certain pan-allergens in edible insects, such as tropomyosin and arginine kinase, as well as other specific allergens such as chitin, chitinase and paramyosin. The available literature suggests that certain food processing technologies (e.g. chemical or enzymatic hydrolysis, heat treatment or ultrasonic treatment) may reduce sensitisation, but further data are needed. It is also important to note that certain cross-allergens, such as gluten, mustard and celery may be present in insects, which can be introduced through feed and the environment, so monitoring these is also a priority for food manufacturers.

Edible insects are also promising from a sustainability and environmental point of view, as their production has a lower impact on the environment than other livestocks: their land and water requirements are lower, they can be fed with a wide range of agricultural, food and household kitchen waste, they have minimal greenhouse gas, and ammonia emissions compared to cattle or pigs and have high reproduction and feed conversion rates.

In the European Union *Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001*, considers edible insects and foods produced with edible insects as *novel foods*. Under the Regulation, four species of insects are currently included in the EU list of novel foods, which can be placed on the market in different forms by applicants:

- dried *Tenebrio molitor* larva,
- frozen, dried and powder forms of *Locusta migratoria,*
- frozen, dried and powder forms of yellow mealworm (*Tenebrio molitor* larva),
- frozen, dried and powder forms of *Acheta domesticus,*
- *Acheta domesticus* (house cricket) partially defatted powder,
- frozen, paste, dried and powder forms of *Alphitobius diaperinus* larvae (lesser mealworm).

Regulations of the European Union are also valid and in force in Hungary, therefore the listed authorised ingredients and products can be placed on the market in Hungary. However, *FM Regulation 36/2014 (XII.17.) on food information* provides specific labelling requirements for foods containing insect protein at the Member State level.

In Europe and in Hungary, low consumer acceptance is one of the barriers to the introduction of insects into the human diet, therefore consumer research on edible insects usually focuses on willingness to consume. Literature data suggest that positive influencing factors include knowledge of beneficial nutritional properties and environmental impact, previous positive experiences, curiosity and sensation seeking. The main reasons for rejection are *food neophobia* and disgust, especially for whole insects. Food neophobia is the fear of new food types and food products and is prevalent in Central Europe and Hungary. Disgust is due to the fact that in Western culture insects are associated with contamination and a lack of hygiene. The cultural effect on acceptance is also reflected at national levels: there are also significant differences between European countries. In Italy, for example, consumer acceptance is low, which may be mainly due to the strong gastronomic traditions which are also present in Hungary. The available literature suggests that in Europe, education and the introduction of insects in "invisible forms" may be a solution, as rejection is much lower when the consumer cannot see certain parts of insects (e.g. head, eyes, legs).

Many studies on the potential use of edible insects as food have been published over the past decade. Many product groups have been included in development processes, but most of the publications are related to cereal-based products: breads, biscuits, dry pasta containing edible insects have been developed and marketed. To these products, edible insects can be added simply, even in "invisible forms" (e.g. as powders), thus increasing the protein content of the finished products and improving their consumer acceptance. Among meat products, minced meat-based preparations, cold cuts, sausages, and meat substitutes containing other alternative proteins appear to be the most promising to enrich, but insect-based milk replacers are also being experimented with. However, snack products, including energy and protein bars, sauces, condiment products, insect oils and even 3D printed insect foods, are also appearing in the international literature and on store shelves.

The main objective of my doctoral thesis was to identify the factors influencing consumer acceptance of foods containing edible insects, the factors that can improve acceptance, and the potential applications of insects in food product development. In order to achieve my research aims, I evaluated and compared powders of several edible insect species from sensory, techno-functional and nutritional perspectives. For the comparison, I aimed to map the composition and sensory properties of the insect powders and to determine the consumer acceptance and preference of foods developed with these powders, even by enriching food products for special nutritional needs and using modern methods that provide more informative results than traditional consumer sensory tests. I also aimed to investigate the impact of insect powders as ingredients on several techno-functional properties, the nutritional composition and sensory properties of the products developed. I grouped my detailed research objectives according to the evaluated products:

Insect powders:

- determination of the proximate composition (dry matter content, moisture content, ash content, protein content, fat content, fibre content, nitrogen-free extract content) of insect powders,
- characterisation and classification of insect powders using FT-NIR spectroscopy,
- training of assessors (panelists) to carry out descriptive sensory evaluation of insect powders,
- create a list of sensory attributes of insect powders using the trained panel,
- sensory profiling of the evaluated insect powders.

Insect-enriched foods:

- analysis of mixtures of insect powder and wheat flour using FT-NIR spectroscopy,
- development of a bakery product enriched with insect powder and evaluating certain techno-functional properties and quality parameters of the product,
- chemometric and sensometric evaluation of a dry pasta product enriched with insect powder,
- consumer sensory evaluation of bakery and pasta products enriched with insect powders, identification of factors and product characteristics influencing consumer acceptance and preference.

2. MATERIALS AND METHODS

During the research, I examined seven internationally commercially available insect powders and food products enriched with them. Using each of the seven insect powders, I prepared insect powder and wheat flour mixtures in a ratio of 5-50% powder. I developed a scottish shortbread-type bakery product recipe based on oat and buckwheat flour, enriched with 0-15% *Acheta domesticus* powder. The research also included the chemo- and sensometric evaluation of buckwheat flour-based dry pasta products enriched with 0-10% *Bombyx mori* powder, which were made during a previous product development work (Figure 1).

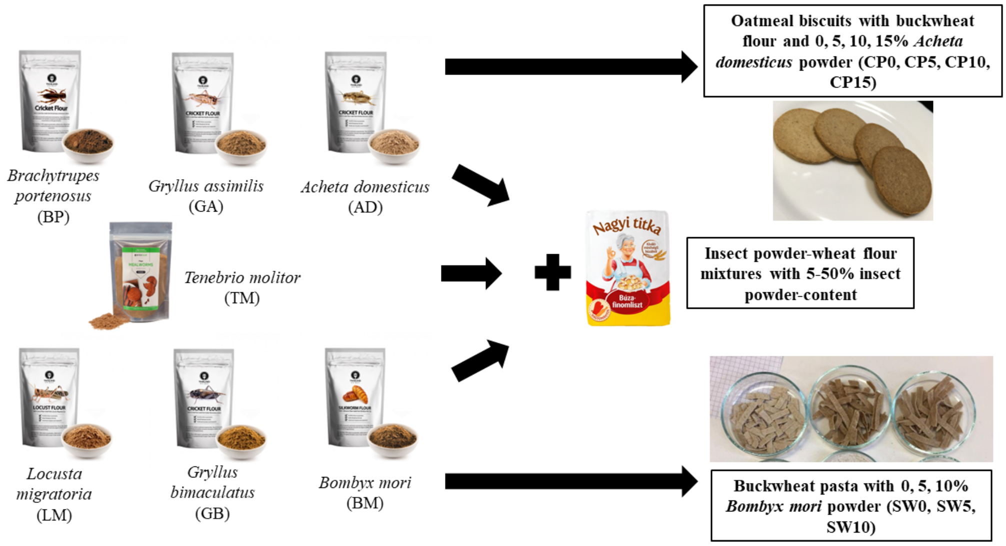

Figure 1: The evaluated insect powder products and the prepared food product samples.

During the research, I determined and compared the proximate composition of the seven insect powders (*Acheta domesticus, Tenebrio molitor, Locusta migratoria, Gryllus assimilis, Gryllus bimaculatus, Brachytrupes portentosus, Bombyx mori*) in the accredited laboratory of Eurofins Food and Feed Testing Budapest Kft. (formerly Wessling Hungary Kft.) using the following analytical methods:

- Determination of dry matter and moisture content: gravimetric method,
- Determination of protein content: Dumas method,
- Determination of fat content: gravimetric method after acid digestion and extraction,
- Determination of crude fibre content: according to MSZ 3626:1986 standard,
- Determination of ash content: gravimetric method,
- Determination of the nitrogen-free extract content: by calculation.

For the spectral data acquisition of the insect powders, I used FT-NIR spectroscopy with diffuse reflectance measurement setup. For sensory profiling, I performed a quantitative sensory profile analysis of the powders using a trained sensory panel of 15 panelists according to the relevant standard MSZ EN ISO 13299:2010 standard.

Using the seven insect powders and wheat flour, I prepared mixtures with insect powder ratio of 5-50% in 5% steps and also analysed the mixtures using FT-NIR spectroscopy with diffuse reflectance measurement setup.

I developed a recipe for a gluten- and lactose-free, oat- and buckwheat flour-based biscuit product containing different amounts (0, 5, 10 and 15%) of *Acheta domesticus* powder, and performed a complex evaluation of the test results of a previously developed buckwheat flour-based dry pasta product containing different amounts (0, 5, 10%) of *Bombyx mori* powder. For both products, some relevant techno-functional properties and quality parameters were determined:

- Oat biscuit products with AD powder:
    - colour measurement in three-dimensional (L* a* b*) colour space,
    - determination of total titratable acidity (according to MSZ 20501-1:2007 standard),
    - instrumented texture analysis – hardness: three-point bending test with texture analyser,

- Buckwheat pasta products with BM powder:
    - colour measurement in three-dimensional (L* a* b*) colour space,
    - determination of moisture content (according to Directive 2-321 of Codex Alimentarius Hungaricus),
    - determination of total titratable acidity (according to MSZ 6369-11:1987 standard),
    - cooking time, water absorption (swelling capacity), determination of the percentage of overcooked pieces (according to MSZ 20500-1:1985 standard and Directive 2-321 of Codex Alimentarius Hungaricus).

In order to determine the consumer preference and liking variables of the developed products and the factors influencing them, consumer sensory analysis was conducted according to MSZ EN ISO 11136:2017 standard, using 9-point hedonic scales and advanced sensory testing methods:

- Oat biscuit products with AD powder:
    - Check-All-That-Apply (CATA) analysis: multiple-choice questionnaire, using which panelists select which of the predefined attributes and properties they think are present in the sample,
    - determinates the *’drivers of liking’*: attributes and properties that positively influence the liking of products,
    -
- Buckwheat pasta products with BM powder:
    - Collecting *’Just-About-Right’* data on optimum scales, three main points on the scales: not intensive enough - just right - too intensive,
    - *Penalty analysis* based on the data: weaknesses and strengths of the products, identification of the properties to be improved.

To analyse and interpret the results, I used univariate and multivariate statistical methods at 5% significance level:

- Kruskal-Wallis test and Analysis of Variance (ANOVA):
    - with Conover-Iman és Tukey post hoc tests,
- Principal Component Analysis (PCA),
- Pre-processing methods of the NIR spectra:
    - Standard Normal Variate (SNV),
    - Derivatives,
- Hierarchical Cluster Analysis (HCA),
- Multiple Factor Analysis (MFA),
- Linear Discriminant Analysis (LDA),
- Partial Least Squares Regression (PLSR),
- Pearson correlation,
- Cochran's Q test,
- Correspondence Analysis (CA),
- Principal Coordinate Analysis (PCoA),
- One sample *t*-test.

3. RESULTS AND DISCUSSION

There were significant differences in **the proximate composition of the examined insect powders** according to the Kruskal-Wallis tests ($p<0.05$). The largest differences were observed in the fat content, which was the lowest for LM and the highest for TM. As the commonly used nitrogen-protein conversion factor of 6.25 for the protein content determination of animal products overestimates insects' protein content by 17% due to the nitrogen content of chitin, a modified nitrogen-protein conversion factor (k_p=4.97) calculated based on the available literature data was used to determine the protein content, which may be a more accurate way to estimate of the protein content of insects based on nitrogen content. The BP powder had the highest measured and calculated (raw and true) protein content on a dry matter basis (true protein content: 58.53 g/100 g d.m.). With the new conversion factor I calculated, the true protein content differs by 20.48% from the measured raw protein content, which allows a more accurate value to be obtained.

The TM sample had the highest fat content (fat content: 29.65 g/100 g d.m.), while the LM sample had the highest crude fibre content (crude fibre content: 12.36 g/100 g d.m.).

The biplot (Figure 2) generated by principal component analysis (PCA) makes it easy to visualise the correlations between the samples and the proximate composition results: the closer two points are located to each other in the plot, the greater the correlation between them. The figure clearly shows that the TM and BM samples are well separated from the other samples. The reason is that these two powders are from one larval and one pupae stage insect, and therefore have a higher fat content and lower protein content than the other powders made of adult insects.

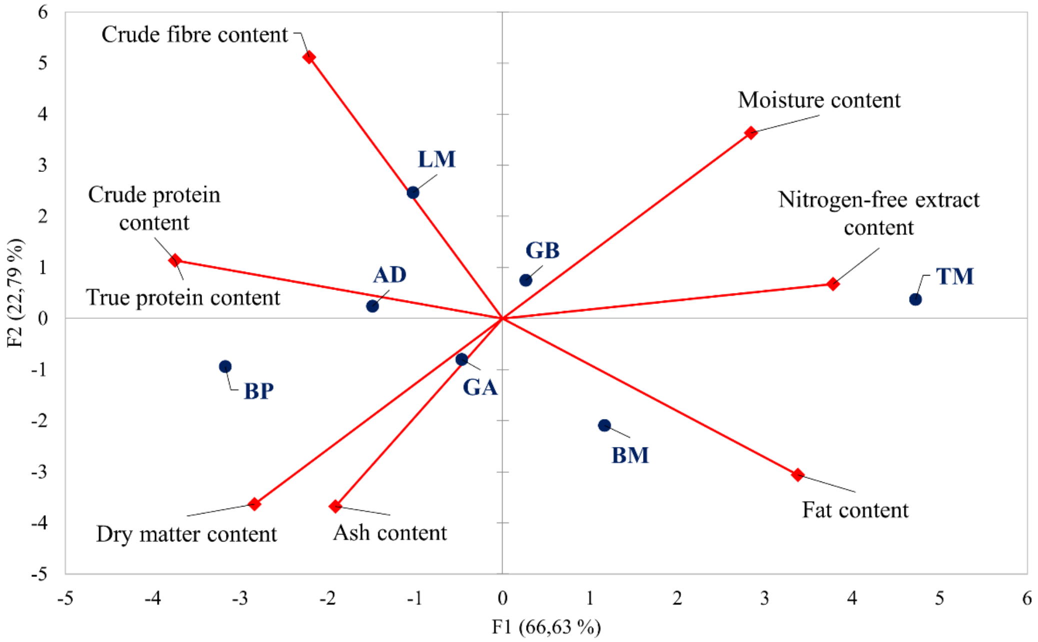

Figure 2: A biplot generated by principal component analysis (PCA) on the dry matter based proximate composition data of the examined insect powders. (n=7, F1 + F2 = 89,41%). AD: *Acheta domesticus*, TM: *Tenebrio molitor*, LM: *Locusta migratoria*, GA: *Gryllus assimilis*, GB: *Gryllus bimaculatus*, BP: *Brachytrupes portentosus*, BM: *Bombyx mori.*

Based on the second derivative **FT-NIR spectra of the insect powders**, the studied insect powders are mainly composed of proteins, fats and carbohydrates, and the absorption peaks associated with these types of compounds can be clearly observed in Figure 3. The 12500-9000 cm-1 wavenumber range is most affected by the colour of the powders and has therefore been excluded from the analysis. The spectral properties of the examined powders are generally similar, with major differences observed in the 6000-5500 cm^{-1} and 5300-5000 cm^{-1} wavenumber ranges. These differences may be due to the different composition of the samples tested, mainly the fat and protein content. A notable difference is presented in the 5917 cm^{-1} absorption band, which may indicate the presence of aromatic and sulphur-containing amino acids. This is clearly visible in the case of LM and AD powders, while it can be identified as a shoulder in the spectra of the other samples analysed.

The TM and BM species belong taxonomically to two different orders, and their powders' spectra are more distinct from the other samples. The hierarchical cluster analysis (HCA) performed has also placed these samples in separate clusters (Figure 4). This is probably due to the different developmental stages, i.e. larval and pupal stage, and the resulting compositional differences, rather than taxonomic classification.

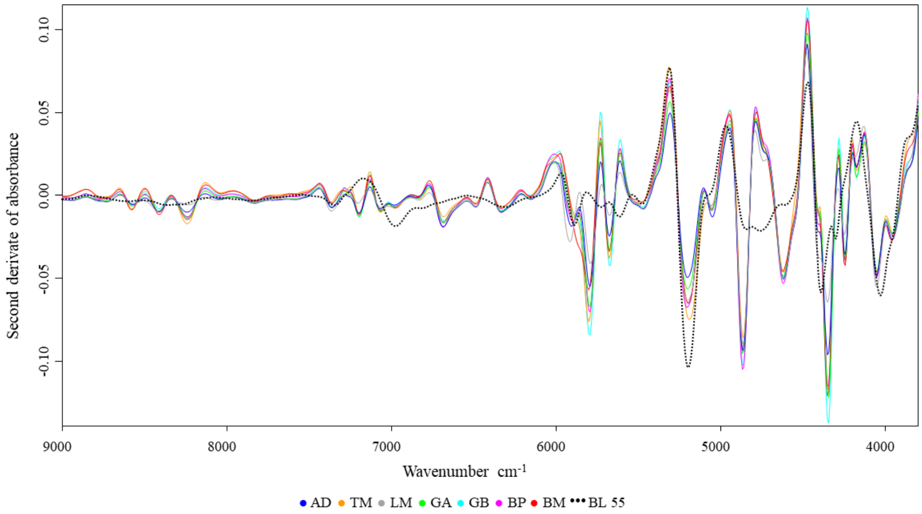

Figure 3: Second-derivative NIR spectra of the insect powders and BL 55 wheat flour after SNV data pre-processing (n=8). AD: *Acheta domesticus*, TM: *Tenebrio molitor*, LM: *Locusta migratoria*, GA: *Gryllus assimilis*, GB: *Gryllus bimaculatus*, BP: *Brachytrupes portentosus*, BM: *Bombyx mori.*

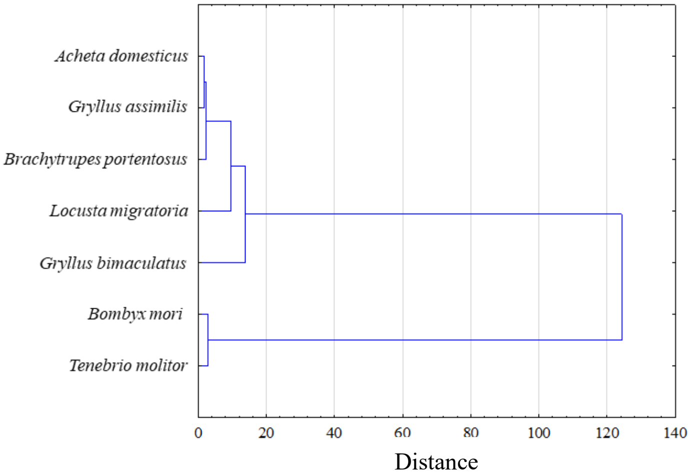

Figure 4: Dendrogram of the examined insect powders, obtained by hierarchical cluster analysis (HCA), squared Euclidean distance and Ward's method (n=7).

Based on the results of the **sensory profile analysis of the insect powders**, the samples are generally similar, but differ greatly in several characteristics. In the spider web plot (Figure 5), which is based on the results obtained, the lines of the samples tend to follow each other, but differences in intensity are observed for almost all attributes.

Based on the ANOVA performed, the insect powders differed from each other on a statistically significant level ($p<0.05$) for 25 of the 27 sensory attributes identified by the panel's consensus: the two exceptions are the cereal odour and the sweet taste. The powders were characterized by a moderately intense brown colour and mouthcoating effect, with the most intense sensory attributes being global odour and flavour intensity, seedy odour and flavour, cereal odour and flavour, toasty odour and flavour, and persistent taste. The major differences in appearance and texture of the TM sample are due to the different particle size, as this powder came from a different manufacturer who used a different degree of grinding.

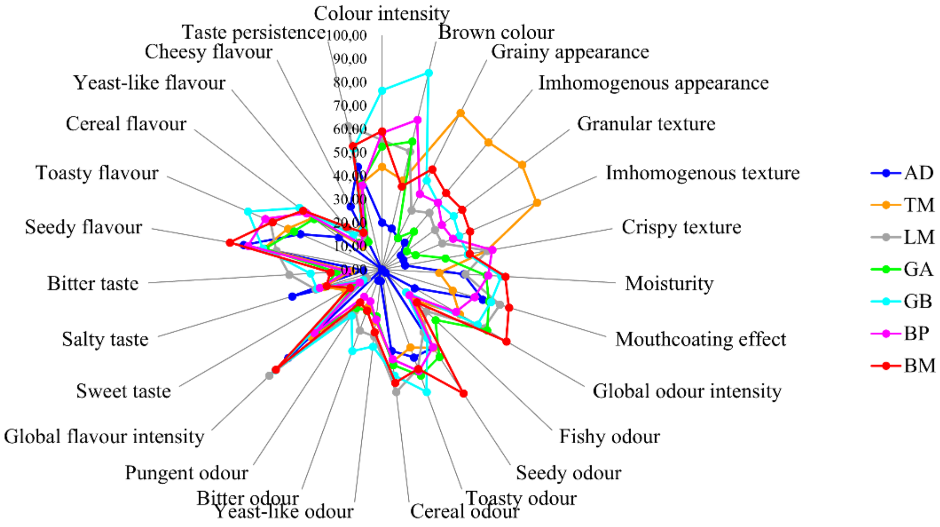

Figure 5: Sensory profiles of the tested insect powders based on 27 attributes identified by a trained panel’s consensus (mean, n=7). AD: *Acheta domesticus*, TM: *Tenebrio molitor*, LM: *Locusta migratoria*, GA: *Gryllus assimilis*, GB: *Gryllus bimaculatus*, BP: *Brachytrupes portentosus*, BM: *Bombyx mori.*

From the biplot generated by PCA performed on the results, it can be observed that the AD insect powder – which was used as a reference sample – is located further away from the other samples, in the negative regions of the x and y axis (Figure 6). This may be due to the relatively low scores for almost all attributes, i.e. the panelists rated this powder as the least intense. A short distance can be observed between the BM, GB and LM powders and these samples are close to the general and more specific flavour and odour attributes, suggesting that these samples had the most intense odour and flavour. The more specific odour and flavour characteristics and the global odour and flavour intensity are also close to each other, suggesting that when these specific characteristics were felt more intensely by the panelists, they gave higher scores for global odour intensity and global flavour intensity.

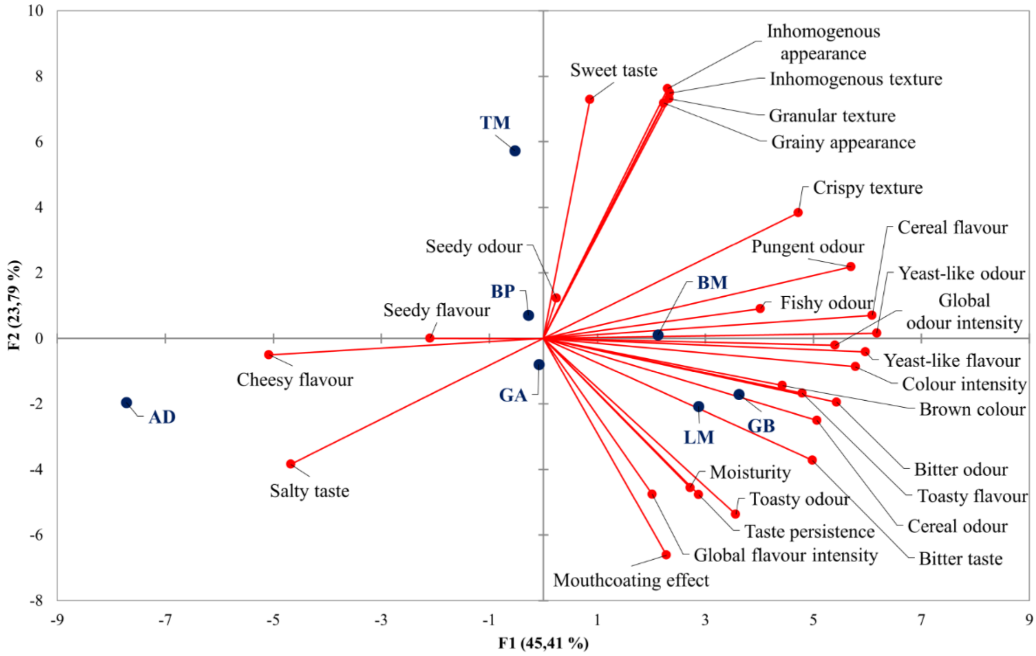

Figure 6: The biplot generated by principal component analysis (PCA) run on the sensory profile data of the insect powders examined (n=7, F1 + F2 = 69,20%). AD: *Acheta domesticus*, TM: *Tenebrio molitor*, LM: *Locusta migratoria*, GA: *Gryllus assimilis*, GB: *Gryllus bimaculatus*, BP: *Brachytrupes portentosus*, BM: *Bombyx mori.*

When analysing the **NIR spectra of the insect powder-wheat flour mixtures**, it was observed that the spectral differences increased with the increasing amounts of insect powder added to the mixture. The PCA score plot in Figure 7 shows that the mixtures differ not only in terms of the different insect species but also in terms of the mixing levels, with the latter being separated along the F1 principal component, while the different species are separated along the F2 principal component. The mixtures containing TM and BM powders also show similarities here: they are more distant from the other samples, the F2 principal component is negative and the F1 principal component is more in the positive range and along the latter, they 'overlap'.

Linear discriminant analysis classified the mixtures with an accuracy of 98.35% after leave-seven-out cross-validation of the models. The LDA diagram (Figure 8) also shows that the BM and TM powders are very similar, the overlap is due to the similarities in the shape of the characteristic peaks of the spectra of these insect powders, which in this case is also due to the specificities of the larval and pupal developmental stages.

For the models developed for the quantitative estimation, the PLSR model using the spectra preprocessed using the SNV method and removing the water absorption peaks proved to be the most efficient, with a model obtained an R^2 value of 0.999 (Table 1).

Pre-processing	**Spectral range (cm^{-1})**	**R^2 value**	**RMSEE [%]**	**Q^2**	**RMSECV [%]**	**Bias**	**RPD**	**Rank**
SNV	9000-7313, 6557-5971, 4173-3857	0,999	0,52	0,998	0,65	-0,0342	21	10

Table 1: The key statistical parameters of the most efficient PLSR model for insect powder-wheat flour mixtures.

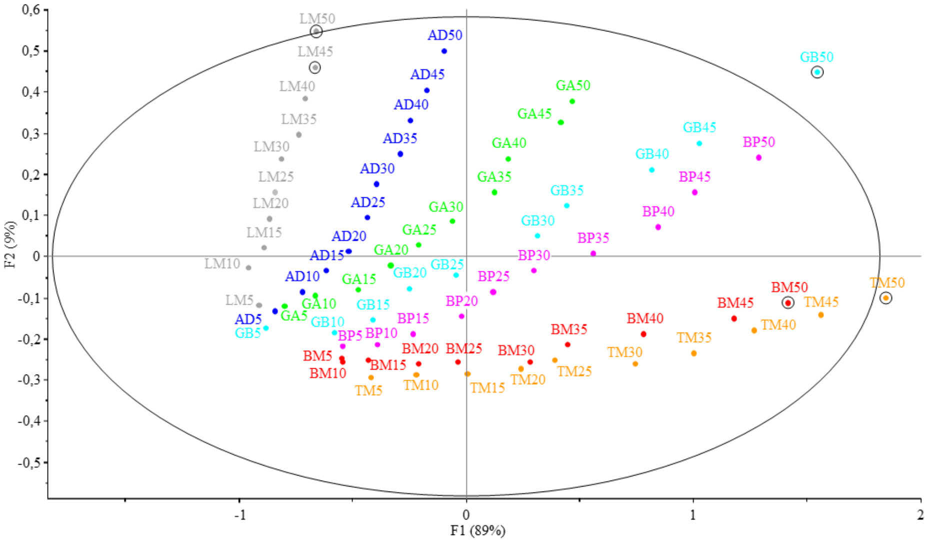

Figure 7: Score plot generated by principal component analysis (PCA) performed on the raw spectral data of the insect powder-wheat flour mixtures (n=70, F1 + F2 = 98%).

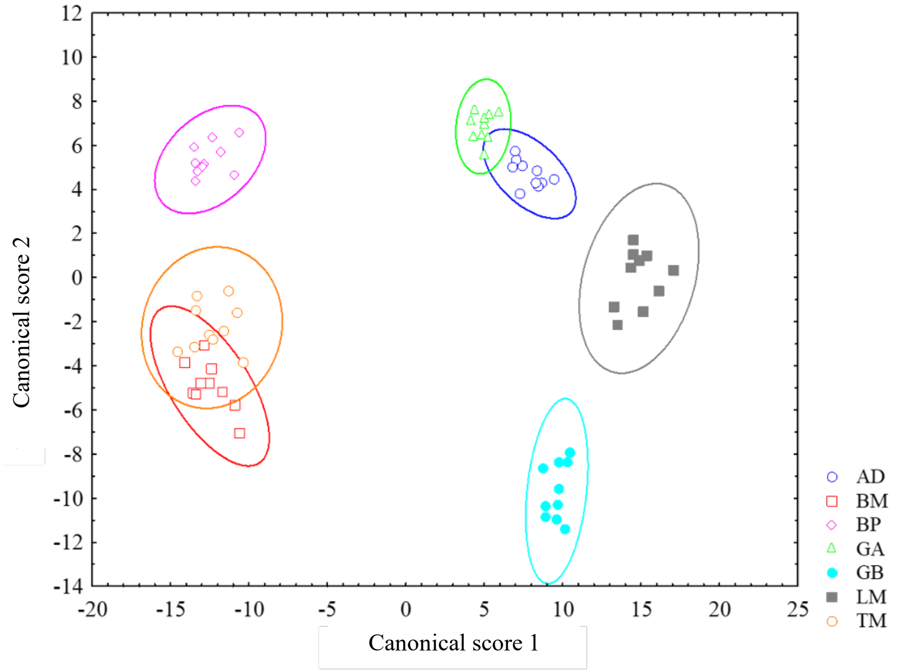

Figure 8: Linear discriminant analysis diagram of the PCA-generated principal component scores run on the spectral data of insect powder-wheat flour mixtures (5-50%) (n=70).

A challenge in the development of the **oat biscuits enriched with AD powder** (Figure 9) made from gluten- and lactose-free ingredients, was that the light-coloured oatmeal was very much darkened by the insect powder, therefore the sensory evaluation would have been greatly affected by the colour of the samples (e.g. it would have been clear to consumers which was the control sample). As a solution, darker coloured buckwheat flour was added to the dough to blunt the differences. During the test baking sessions, I achieved the best results with 20% buckwheat flour.

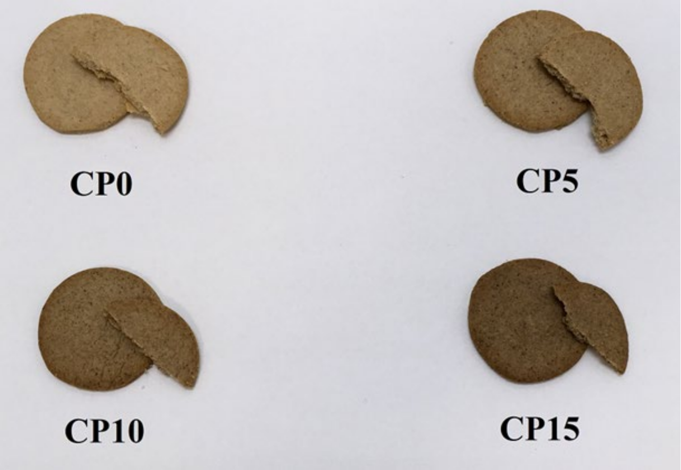

Figure 9: Appearance of the control sample and the insect powder-enriched biscuits.

Based on the nutrient content calculation, the insect powder slightly increases the energy value and the fat content, decreases the carbohydrate content and increases the protein content (Table 2). If the developed oatmeal biscuits were marketed as pre-packaged products, biscuits containing 10 and 15% insect powder could bear the claim 'source of protein' under Regulation (EC) No 1924/2006 of the European Parliament and of the Council on nutrition and health claims made on foods, as their energy value from protein exceeds 12%. The fibre content of biscuits is slightly reduced by the powder, but all three enriched products could still bear the claim 'high fibre' under Regulation (EC) No 1924/2006, as their dietary fibre content exceeds 6 g/100g.

Table 2: Composition, calculated energy and nutrient content per 100 g of *Acheta domesticus* powder enriched biscuit products. The energy value per 100 g is in bold and the protein/energy values exceeding 12% required to make the claim 'source of protein' under the Annex to Regulation (EC) No 1924/2006 are in bold and in italics.

Sample	Ingredient	Amount (g)	Energy (kcal/100 g)	Fat (g/100 g)	Carbohydrate (g/100 g)	Fibre (g/100 g)	Protein (g/100 g)	Protein/ energy value (%)
CP0	Cricket powder	0,00	0,00	0,00	0,00	0,00	0,00	9,46
	Oat flour	51,88	191,96	4,15	28,53	5,71	7,26	
	Buckwheat flour	12,97	43,32	0,13	9,16	1,30	1,63	
	Lactose free butter	21,98	157,63	17,83	0,13	0,00	0,19	
	Lactose free sour cream	13,16	17,77	1,58	0,45	0,00	0,39	
	Overall	100,00	**410,68**	**23,69**	**38,27**	**7,00**	**9,48**	
CP5	Cricket powder	3,24	14,82	0,59	0,18	0,02	2,20	11,13
	Oat flour	48,64	179,96	3,89	26,75	5,35	6,81	
	Buckwheat flour	12,97	43,32	0,13	9,16	1,30	1,63	
	Lactose free butter	21,98	157,63	17,83	0,13	0,00	0,19	
	Lactose free sour cream	13,16	17,77	1,58	0,45	0,00	0,39	
	Overall	100,00	**413,50**	**24,02**	**36,67**	**6,66**	**11,22**	
CP10	Cricket powder	6,49	29,64	1,18	0,36	0,03	4,40	***12,77***
	Oat flour	45,40	167,96	3,63	24,97	4,99	6,36	
	Buckwheat flour	12,97	43,32	0,13	9,16	1,30	1,63	
	Lactose free butter	21,98	157,63	17,83	0,13	0,00	0,19	
	Lactose free sour cream	13,16	17,77	1,58	0,45	0,00	0,39	
	Overall	100,00	**416,32**	**24,35**	**35,06**	**6,32**	**12,97**	
CP15	Cricket powder	9,73	44,46	1,77	0,54	0,05	6,60	***14,39***
	Oat flour	42,15	155,97	3,37	23,18	4,64	5,90	
	Buckwheat flour	12,97	43,32	0,13	9,16	1,30	1,63	
	Lactose free butter	21,98	157,63	17,83	0,13	0,00	0,19	
	Lactose free sour cream	13,16	17,77	1,58	0,45	0,00	0,39	
	Overall	100,00	**419,14**	**24,68**	**33,46**	**5,98**	**14,71**	

*CP0: biscuits based on flour mix containing 0 g/100 g of AD powder, CP5: biscuits based on flour mix containing 5 g/100 g of AD powder, CP10: biscuits based on flour mix containing 10 g/100 g of AD powder, CP15: biscuits based on flour mix containing 15 g/100 g of AD powder. *The table includes only those ingredients that contains nutrients that provides energy.*

Based on the results of the instrumental measurements and the statistical analyses run on them, the addition of the AD powder to the biscuit significantly changes the colour (mainly the lightness factor (L*)), increases the total titratable acidity of the product, but does not significantly affect the texture.

The liking variables showed that consumers preferred the control and the 5% sample the most, with no significant difference between the two samples for the liking variables for colour, odour and flavour ($p>0.05$). During the CATA analysis, the consumers are asked to select from a predefined list of attributes that they perceive in the product. Correspondence analysis (CA) carried out as part of the analysis showed that the control sample was not intense enough for them, while the 5% product had many positive hedonic indicators, such as friability and cheesy flavour. As the cricket content increases, negative hedonic properties appear, such as too strong, burnt flavour and bitter odour (Figure 10).

From the results of the principal coordinate analysis (PCoA), it can be concluded that of the listed attributes, fattiness, cheesy flavour, toasty odour and friability had a positive influence on the overall liking, as they are close to it and therefore positively correlated. These are called "drivers of liking". The *mean impact analysis*, which is part of the CATA methodology, confirms this result: it calculates the overall liking average for each attribute, both when consumers selected it as present and when as not present. The two values are then subtracted from each other and the difference is the mean impact value. The analysis showed that cheesy flavour, friability and fattiness increased the overall liking, while burnt flavour and brown colour decreased it when they were present in the samples (Figure 11).

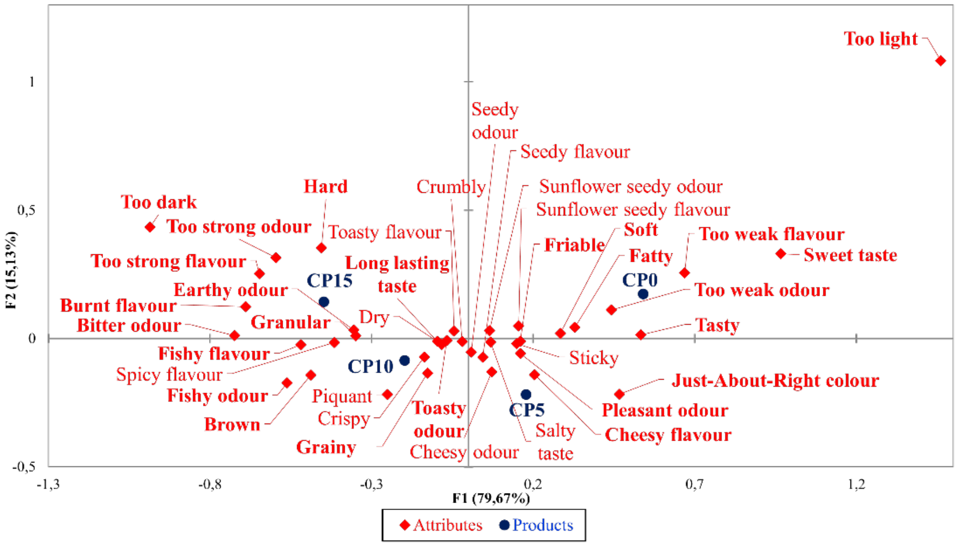

Figure 10: Correspondence analysis of the *Acheta domesticus* powder-enriched biscuit products and CATA questionnaire terms (n=4, F1+F2=94.80%). CP0: biscuits based on flour mix containing 0 g/100 g of AD powder, CP5: biscuits based on flour mix containing 5 g/100 g of AD powder, CP10: biscuits based on flour mix containing 10 g/100 g of AD powder, CP15: biscuits based on flour mix containing 15 g/100 g of AD powder. The terms and attributes that significantly distinguished the biscuit samples tested from each other, as determined by the Cochran Q-test, are highlighted in bold.

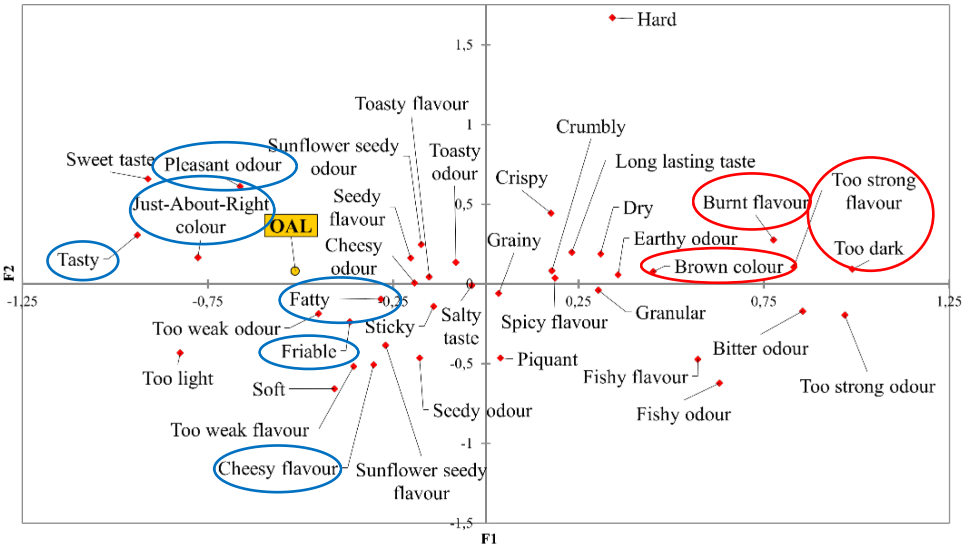

Figure 11: Principal coordinate analysis (PCoA) of the CATA questionnaire terms and the overall liking data used obtained by consumer sensory analysis of the *Acheta domesticus* powder-enriched biscuit products. OAL: Overall Liking.

The challenge in the development of the egg-free, **BM powder enriched buckwheat pasta** products was that it tore very easily during sheeting, so only about 100 g could be produced at a time.

Based on the nutrient content calculation, the insect powder slightly increases the energy value and the fat content, decreases the carbohydrate content and increases the protein content. If the enriched pasta were to be marketed as pre-packaged products, both the control sample and the samples containing the insect powder could bear the claim 'high protein' under Regulation (EC) No 1924/2006, as their energy value from protein is above 20%. The pasta’s fibre content is slightly reduced due to the insect powder addition but could still be labelled with the claim ’high fibre’ (Table 3).

Based on the results of the instrumental measurements and quality parameter tests, and the statistical analyses run on them, the addition of BM powder increases the total titratable acidity of the pasta, reduces its moisture content after drying, reduces its cooking time, increases its water absorption and increases the number of overcooked pieces.

Table 3: Composition, calculated energy and nutrient content of *Bombyx mori* powder-enriched dry pasta products per 100 g of flour mixture, corrected with the moisture content of the pasta. The energy value per 100 g is in bold and the protein/energy values exceeding 20% required to make the 'high protein' claim under the Annex to Regulation (EC) No 1924/2006 are in bold and in italics.

Sample	Ingredient	Amount (g)	Energy (kcal/100 g)	Fat (g/100 g)	Carbohydrate (g/100 g)	Fibre (g/100 g)	Protein (g/100 g)	Protein/ energy value (%)
SW0	Buckwheat flour	80,00	290,40	2,72	57,12	8,00	10,60	***29,24***
	Gluten	20,00	76,40	0,48	2,40	0,00	15,56	
	Silkworm powder	0,00	0,00	0,00	0,00	0,00	0,00	
	Overall**	100,00	**366,80**	**3,20**	**59,52**	**8,00**	**26,16**	
SW5	Buckwheat flour	75,00	272,25	2,55	53,63	7,50	9,94	***31,46***
	Gluten	20,00	76,40	0,48	2,40	0,00	15,56	
	Silkworm powder	5,00	19,48	0,43	1,27	0,30	2,75	
	Overall**	100,00	**368,13**	**3,46**	**57,30**	**7,80**	**28,25**	
SW10	Buckwheat flour	70,00	254,10	2,38	49,98	7,00	9,28	***33,66***
	Gluten	20,00	76,40	0,48	2,40	0,00	15,56	
	Silkworm powder	10,00	38,96	0,85	2,54	0,60	5,50	
	Overall**	100,00	**369,46**	**3,71**	**54,92**	**7,60**	**30,34**	

*SW0: pasta based on a flour mix containing 0 g/100 g of BM powder, SW5: pasta based on a flour mix containing 5 g/100 g of BM powder, SW10: pasta based on a flour mix containing 10 g/100 g of BM powder. *The table includes only those ingredients that contains nutrients that provides energy. **Energy values and macronutrient content have been corrected with the moisture content of the dry pasta.*

Based on the results of the consumer sensory evaluation, the product with 10% insect powder was the most preferred by the assessors, while the product with no insect powder received the lowest liking scores. To collect just-about-right data, an optimum scale is used with 3 main scores: not intense enough (too little), just about right (JAR) and too intense (too much). In the bar charts in Figures 12-14, each product attribute is plotted with the proportion of consumers who responded with that particular response type. The results showed that the control sample was too hard and tasteless. In contrast, the sample with 5% insect content was more acceptable for the consumers, but they found the taste to be even less optimal by the judges. In the case of the 10% insect-containing pasta, the colour was too intense, but the flavour was more optimal for consumers.

Penalty analysis uses *mean drop analysis* to identify the attributes that need to be optimised to increase acceptance, which is very similar to CATA's mean impact analysis. The difference is that the proportion of the responding consumers is also a factor: based on the default settings of the software used, a response rate above 20% is considered relevant. Based on the penalty values obtained, it can be concluded that for sample SW0, colour, global odour intensity, cooked pasta odour and cooked pasta flavour need to be optimised, while for sample SW5, colour, stickiness and cooked pasta flavour, and for sample SW10, global odour intensity and cooked pasta flavour need to be optimised, as these attributes reduced the overall liking the most when they were not optimised according to the assessors.

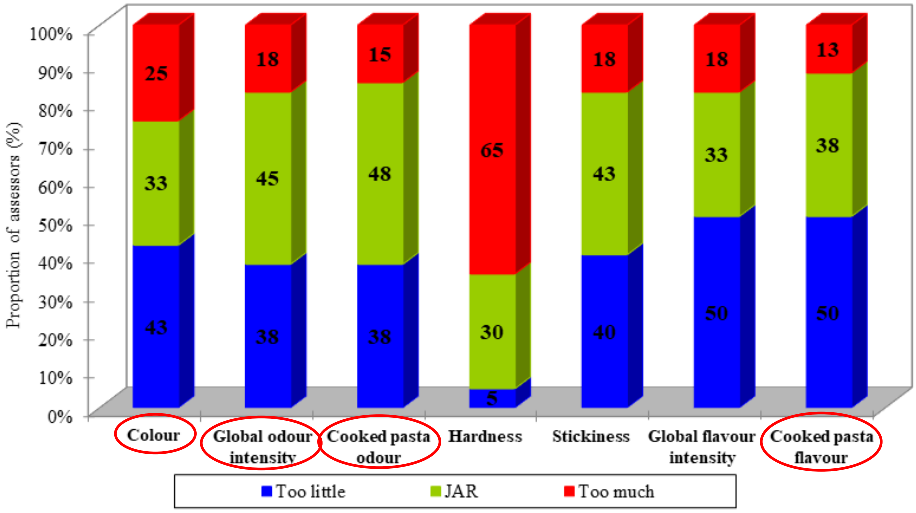

Figure 12: Simplified diagram of the collected JAR data of the dry pasta sample SW0, formed by combining the 9 scale points into three JAR groups with the proportion of the responding assessors.

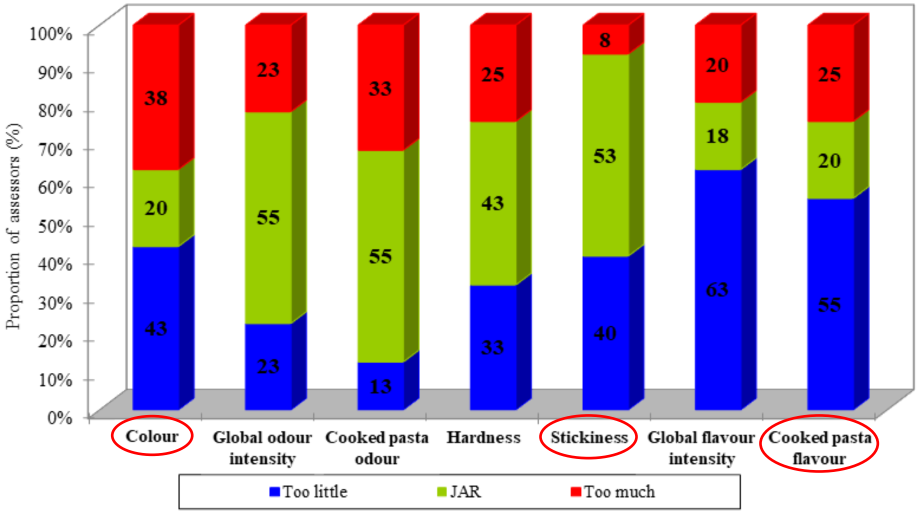

Figure 13: Simplified diagram of the collected JAR data of the dry pasta sample SW5, formed by combining the 9 scale points into three JAR groups with the proportion of the responding assessors.

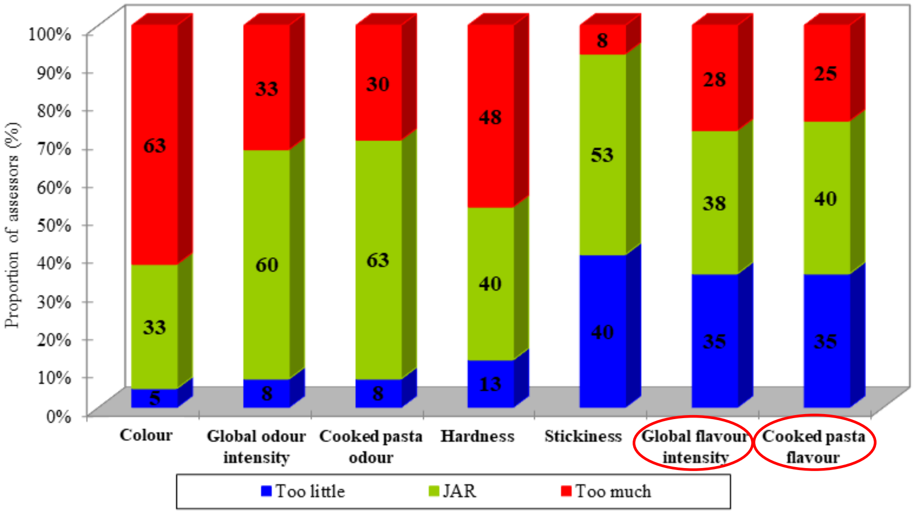

Figure 14: Simplified diagram of the collected JAR data of the dry pasta sample SW10, formed by combining the 9 scale points into three JAR groups with the proportion of the responding assessors.

4. CONCLUSIONS AND RECOMMENDATIONS

Many studies have been carried out on the composition of edible insects, but it is generally observed that researchers only study a few prioritised species (e.g. *Acheta domesticus, Tenebrio molitor, Zophobas morio, Locusta migratoria, Bombyx mori*) and often publish results for only one species. During my research, I examined and compared powders from seven insect species, including species less frequently studied, thus adding to the scientific literature. Using the new nitrogen-protein conversion factor (k_p=4.97) I calculated for insects based on data from the literature will provide more accurate data, but more data from accurate determination of chitin content and amino acid composition is needed to provide a more comprehensive and accurate picture of insects' protein content.

Using the spectral data of insect powders, I developed a chemometric methodology to identify insect species and to estimate the amount of insect powders in wheat flour mixtures with high accuracy. In the future, NIR spectroscopy could be successfully used to detect possible food adulteration and to verify the insect content indicated in product specifications or on labels, even by authorities.

Using quantitative descriptive sensory profile analysis of insect powders, I generated a list of sensory attributes of the powders made of different insect species and determined their sensory profiles. However, an even more accurate picture of the sensory properties of edible insects can be obtained by performing more descriptive sensory tests and consumer sensory evaluation using additional analytical methods, as well as by performing instrumental tests.

By developing and testing a bakery product enriched with *Acheta domesticus* powder and by analysing and evaluating the test results of a dry pasta enriched with *Bombyx mori* powder, I supported the literature conclusions that insect powders are an excellent enrichment ingredient to bakery and pasta products. When added in small amounts, insect powders may either not significantly affect the acceptance and liking of biscuit and pasta products or, in the case of a less popular base product, may even increase them. In addition, these results suggest that edible insect powders can be used for the production and fortification of foods for specific nutritional purposes.

The use of insects in food, as well as further food science and consumer research in the field, will also help food industry innovation, as these ingredients are suitable for food production using modern methods such as 3D food printing. My results also confirm that the methods used can be used to easily and quickly identify sensory attributes that significantly influence consumer preferences and how the products need to be optimised, helping future food product development and easier market introduction of insect-enriched products.

5. NEW SCIENTIFIC RESULTS

1. I generated a list of sensory attributes with a trained panel to determine the sensory profile of seven edible insect species (*Acheta domesticus, Tenebrio molitor, Locusta migratoria, Gryllus assimilis, Gryllus bimaculatus, Brachytrupes portentosus, Bombyx mori*) and determined their sensory profile using quantitative sensory profile analysis. The insect species differed significantly from each other in appearance, texture, odour and flavour, with the most intense sensory properties being global odour intensity, seedy odour, cereal odour, toasty odour, and global flavour intensity, seedy flavour, cereal flavour, toasty flavour and taste persistence, respectively.

Related publication:

***BIRÓ, B.**, GERE, A. (2020): Establishing sensory profiles of edible insect powders. SENV2020_118. In: EUROSENSE 2020 – 9th European Conference on Sensory and Consumer Research, 2020. Rotterdam, The Netherlands, Postillion Convention Centre WTC Rotterdam, 13-16 December 2020.*

2. I was the first to examine edible insect powders and their mixtures with wheat flour using FT-NIR spectroscopy. I developed a chemometric methodology to classify insect powders and their mixtures with wheat flour based on the insect powder content and to estimate the amount of insect powders in the mixtures. The PCA-LDA classification had an accuracy of 98.35% after leave-seven-out cross-validation. The R^2 value of the PLSR model for quantitative prediction of insect powders in the mixtures was 0.999, which was obtained after pre-processing the recorded spectral data with the standard normal variate method.

Related publications:

BENES, E., ***BIRÓ, B.****, FODOR, M., GERE, A. (2022): Analysis of wheat flour-insect powder mixtures based on their near infrared spectra. In: Food Chemistry: X, 100266.* ***IF$_{2023}$ = 6,1, SJR indicator: D1***

GERE, A., ***BIRÓ, B.*** *(2023): Alternatív fehérjeforrások: Lehetőségek és korlátok. In: Hungalimentaria 2023 – XIV. Hungalimentaria Élelmiszer-biztonsági Konferencia és Kiállítás, 2023. Budapest: Aquaworld Resort Budapest Hotel, 18-19 April 2023.*

3. I have developed a new recipe for a gluten- and lactose-free bakery product containing different amounts (0, 5, 10 15%) of *Acheta domesticus* powder. Using instrumental methods, I examined the colour of the developed biscuit products, determined their hardness (control sample: 160.22±43.91, sample containing 15% *Acheta domesticus* powder: 183.72±40.79) and total titratable acidity (control sample: 9.95±0.35, sample containing 15% *Acheta domesticus* powder: 17.65±0.35), and calculated their energy value and macronutrient content. I also determined the consumer preference and liking of the products.

Related publications:

BIRÓ, B.*, SIPOS, M. A., KOVÁCS, A., BADAKNÉ KERTI, K., PÁSZTORNÉ HUSZÁR, K., GERE, A. (2020): Cricket-Enriched Oat Biscuit: Technological Analysis and Sensory Evaluation. In: Foods, 9 (11) 1561.* ***IF_{2023} = 5,2, SJR indicator: Q1***

GERE, A., BAJUSZ, D., ***BIRÓ, B.****, RÁCZ, A. (2021): Discrimination Ability of Assessors in Check-All-That-Apply Tests: Method and Product Development. In: Foods, 10 (5) 1123.* ***IF_{2023} = 5,2, SJR indicator: Q1***

BIRÓ, B.*, HÉBERGER, K., BADAK-KERTI, K., SIPOS, M. A., PÁSZTOR-HUSZÁR, K., GERE, A. (2019): Cricket powder enriched oat biscuit. Sensory and technological evaluation. Abstract: 422. In: PANGBORN 2019 – 13th Pangborn Sensory Science Symposium 2019. Edinburgh, United Kingdom, Edinburgh International Conference Centre (EICC), 28 July – 1 August 2019.*

BIRÓ, B.*, SIPOS, M. A., KOVÁCS, A., BADAK-KERTI, K., PÁSZTOR-HUSZÁR, K., GERE, A. (2019): Sensory evaluation of cricket-enriched oat biscuits using check-all-that-apply analysis. P02. In: HÉBERGER, K. (Szerk.): Conferentia Chemometrica 2019. Karcag, Hungary, Hotel Nimród, 8-12 September 2019.*

GERE, A., ***BIRÓ, B.*** *(2023): Alternatív fehérjeforrások: Lehetőségek és korlátok. In: Hungalimentaria 2023 – XIV. Hungalimentaria Élelmiszer-biztonsági Konferencia és Kiállítás, 2023. Budapest: Aquaworld Resort Budapest Hotel, 18-19 April 2023.*

4. Based on the evaluation of the results of instrumental and consumer sensory evaluation of *Acheta domesticus* powder-enriched biscuit products and *Bombyx mori* powder-enriched dry pasta products, I have demonstrated that insect powders are excellent enrichments to bakery and pasta products: their high-quality protein content increases (protein content of *Acheta domesticus* powder-enriched biscuit products: 9,48 to 14,71 g/100 g, protein content of pasta products containing *Bombyx mori* powder: 23,71 to 27,71 g/100 g), without a significant increase of their energy value (energy value of biscuit products containing *Acheta domesticus* powder: 410,68 to 419,14 kcal/100 g, energy content of pasta products containing *Bombyx mori* powder: 332,43 to 337,50 kcal/100 g). The manufacturing process of the products is not significantly influenced by the addition of the powders, but they have a statistically significant effect on some properties and attributes of the finished products. Among the sensory properties of the products developed, the texture is of particular importance, which is not or only slightly influenced by the insect powders and can even be improved by small amounts of these enrichments, according to my research results.

Related publications:

BIRÓ, B.**, FODOR, R., SZEDLJAK, I., PÁSZTOR-HUSZÁR, K., GERE, A. (2019): Buckwheat-pasta Enriched with Silkworm Powder: Technological Analysis and Sensory Evaluation. In: LWT – Food Science and Technology, 116 108542.* ***IF$_{2023}$ = 6, SJR indicator: D1

BIRÓ, B.**, SIPOS, M. A., KOVÁCS, A., BADAKNÉ KERTI, K., PÁSZTORNÉ HUSZÁR, K., GERE, A. (2020): Cricket-Enriched Oat Biscuit: Technological Analysis and Sensory Evaluation. In: Foods, 9 (11) 1561.* ***IF$_{2023}$ = 5,2, SJR indicator: Q1

***BIRÓ, B.**, HÉBERGER, K., BADAK-KERTI, K., SIPOS, M. A., PÁSZTOR-HUSZÁR, K., GERE, A. (2019): Cricket powder enriched oat biscuit. Sensory and technological evaluation. Abstract: 422. In: PANGBORN 2019 – 13th Pangborn Sensory Science Symposium 2019. Edinburgh, United Kingdom, Edinburgh International Conference Centre (EICC), 28 July – 1 August 2019.*

***BIRÓ, B.**, SIPOS, M. A., KOVÁCS, A., BADAK-KERTI, K., PÁSZTOR-HUSZÁR, K., GERE, A. (2019): Sensory evaluation of cricket-enriched oat biscuits using check-all-that-apply analysis. P02. In: HÉBERGER, K. (Szerk.): Conferentia Chemometrica 2019. Karcag, Hungary, Hotel Nimród, 8-12 September 2019.*

BIRÓ, B., *PÁSZTOR-HUSZÁR, K., GERE, A. (2021): Insect-enriched food products from a nutritional point of view. PANG2021_0194. In: PANGBORN 2021 – 14th Pangborn Sensory Science Symposium, 2021. Online Conference, 9-12 August 2021.*

5. I was the first in Hungary to use modern consumer sensory analysis methods (Check-All-That-Apply (CATA) analysis, Penalty analysis) to develop bakery and pasta products containing insect powders and to determine the product characteristics that influence the acceptance and liking of the developed products. I demonstrated that the addition of small amounts (5-10%) of insect powders increases or does not significantly affect the liking scores of oat and buckwheat flour-based biscuit and pasta products, while larger amounts decrease the liking scores due to their intense colour, odour and flavour.

Related publications:

BIRÓ, B.*, FODOR, R., SZEDLJAK, I., PÁSZTOR-HUSZÁR, K., GERE, A. (2019): Buckwheat-pasta Enriched with Silkworm Powder: Technological Analysis and Sensory Evaluation. In: LWT – Food Science and Technology, 116 108542.* ***IF$_{2023}$ = 6, SJR indicator: D1***

BIRÓ, B.*, SIPOS, M. A., KOVÁCS, A., BADAKNÉ KERTI, K., PÁSZTORNÉ HUSZÁR, K., GERE, A. (2020): Cricket-Enriched Oat Biscuit: Technological Analysis and Sensory Evaluation. In: Foods, 9 (11) 1561.* ***IF$_{2023}$ = 5,2, SJR indicator: Q1***

BIRÓ, B.*, HÉBERGER, K., BADAK-KERTI, K., SIPOS, M. A., PÁSZTOR-HUSZÁR, K., GERE, A. (2019): Cricket powder enriched oat biscuit. Sensory and technological evaluation. Abstract: 422. In: PANGBORN 2019 – 13th Pangborn Sensory Science Symposium 2019. Edinburgh, United Kingdom, Edinburgh International Conference Centre (EICC), 28 July – 1 August 2019.*

BIRÓ, B.*, SIPOS, M. A., KOVÁCS, A., BADAK-KERTI, K., PÁSZTOR-HUSZÁR, K., GERE, A. (2019): Sensory evaluation of cricket-enriched oat biscuits using check-all-that-apply analysis. P02. In: HÉBERGER, K. (Szerk.): Conferentia Chemometrica 2019. Karcag, Hungary, Hotel Nimród, 8-12 September 2019.*

BIRÓ, B.*, PÁSZTOR-HUSZÁR, K., GERE, A. (2021): Insect-enriched food products from a nutritional point of view. PANG2021_0194. In: PANGBORN 2021 – 14th Pangborn Sensory Science Symposium, 2021. Online Conference, 9-12 August 2021.*

GERE, A., ***BIRÓ, B.*** *(2023): Alternatív fehérjeforrások: Lehetőségek és korlátok. In: Hungalimentaria 2023 – XIV. Hungalimentaria Élelmiszer-biztonsági Konferencia és Kiállítás, 2023. Budapest: Aquaworld Resort Budapest Hotel, 18-19 April 2023.*

6. PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

Articles in journals with impact factor:

BIRÓ, B., FODOR, R., SZEDLJAK, I., PÁSZTOR-HUSZÁR, K., GERE, A. (2019): Buckwheat-pasta Enriched with Silkworm Powder: Technological Analysis and Sensory Evaluation. In: *LWT – Food Science and Technology,* 116 108542. **IF$_{2023}$ = 6, SJR indicator: D1**

BIRÓ, B., SIPOS, M. A., KOVÁCS, A., BADAKNÉ KERTI, K., PÁSZTORNÉ HUSZÁR, K., GERE, A. (2020): Cricket-Enriched Oat Biscuit: Technological Analysis and Sensory Evaluation. In: *Foods*, 9 (11) 1561. **IF$_{2023}$ = 5,2, SJR indicator: Q1**

GERE, A., BAJUSZ, D., **BIRÓ, B.**, RÁCZ, A. (2021): Discrimination Ability of Assessors in Check-All-That-Apply Tests: Method and Product Development. In: *Foods,* 10 (5) 1123. **IF$_{2023}$ = 5,2, SJR indicator: Q1**

BENES, E., **BIRÓ, B.**, FODOR, M., GERE, A. (2022): Analysis of wheat flour-insect powder mixtures based on their near infrared spectra. In: *Food Chemistry: X,* 100266. **IF$_{2023}$ = 6,1, SJR indicator: D1**

International conference summaries:

BIRÓ, B., PÁSZTOR-HUSZÁR, K., GERE A. (2018): Consumer acceptance of insect-based foods. 78-79. p. In: DALMADI, I., BARANYAI; L., DUC NGUYEN, Q. (Szerk): *3rd FoodConf – Third International Conference on Food Science and Technology, 2018.* Budapest, Hungary, Szent István Egyetem, Élelmiszertudományi Kar, 29 November – 1 December 2018. 178 p.

BIRÓ, B., HÉBERGER, K., BADAK-KERTI, K., SIPOS, M. A., PÁSZTOR-HUSZÁR, K., GERE, A. (2019): Cricket powder enriched oat biscuit. Sensory and technological evaluation. Abstract: 422. In: *PANGBORN 2019 – 13th Pangborn Sensory Science Symposium 2019.* Edinburgh, United Kingdom, Edinburgh International Conference Centre (EICC), 28 July – 1 August 2019.

BIRÓ, B., SIPOS, M. A., KOVÁCS, A., BADAK-KERTI, K., PÁSZTOR-HUSZÁR, K., GERE, A. (2019): Sensory evaluation of cricket-enriched oat biscuits using check-all-that-apply analysis. P02. In: HÉBERGER, K. (Szerk.): *Conferentia Chemometrica 2019.* Karcag, Hungary, Hotel Nimród, 8-12 September 2019.

BIRÓ, B., GERE, A. (2020): Establishing sensory profiles of edible insect powders. SENV2020_118. In: *EUROSENSE 2020 – 9th European Conference on Sensory and Consumer Research, 2020.* Rotterdam, The Netherlands, Postillion Convention Centre WTC Rotterdam, 13-16 December 2020.

BIRÓ, B., PÁSZTOR-HUSZÁR, K., GERE, A. (2021): Insect-enriched food products from a nutritional point of view. PANG2021_0194. In: *PANGBORN 2021 – 14th Pangborn Sensory Science Symposium*, *2021.* Online Conference, 9-12 August 2021.

Hungarian conference summaries:

BIRÓ, B. (2018): Ehető rovarok táplálkozásélettani jelentősége. 21. p. In: GÁL J., PENKSZA P., PINTÉR R. (Szerk.): *I. Magyar Rovaripari Konferencia, 2018. február 23. Absztraktkötet, Poszterek.* Budapest: Szent István Egyetem, Élelmiszertudományi Kar. 21. p.

BIRÓ, B., PÁSZTORNÉ, HUSZÁR K., GERE, A. (2018): Rovarokkal komplettált élelmiszerek táplálkozástudományi és érzékszervi vizsgálata. 15-16. p. In: SIPOS, L., GERE, A. (Szerk.): *MTA, Kertészeti és Élelmiszertudományi Bizottság, Élelmiszertudományi Albizottság Workshop, 2018. december 6.* Budapest: MTA, Kertészeti és Élelmiszertudományi Bizottság, Élelmiszertudományi Albizottság. 31. p.

GERE, A., **BIRÓ, B.** (2023): Alternatív fehérjeforrások: Lehetőségek és korlátok. In: *Hungalimentaria 2023 – XIV. Hungalimentaria Élelmiszer-biztonsági Konferencia és Kiállítás, 2023.* Budapest: Aquaworld Resort Budapest Hotel, 18-19 April 2023.