Indirect recycling of fast food in insect feed:
UHPLC-MS/MS proteomics to ensure Food safety

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Introduction & Objective

Since 2021, **insect meal** has been **authorised in feed** intended for pigs and poultry, in addition to fish feed. Despite these regulatory changes, the insect meal market remained limited and more expensive due to high production costs.

Recycling wastes as a substrate for insects rearing could be a solution, but the use of former foodstuffs containing **meat or fish** is still **prohibited.**

Is it possible to control?

Currently, there is no validated method to determine whether the substrate on which insects were raised contained banned ingredients.

The objectives of this study were to rear insects on fast food-based substrates & to evaluate an **MS-proteomics approach** for detecting the presence of prohibited residues.

Farm animals → **Food products**

Circular economy

Former foodstuffs

Materials & Methods

MATERIALS:

Reference meats for markers selection:
- Raw + cooked meat
- Industrial meat: beef burger, ham and nuggets)

Substrates:
Poultry feed (Hen) + 10 % (w/w) of:
- Cheese burger 100 % beef
- Pizza "special" with ham & pepperoni
- Chicken nuggets

Insects:
- _Hermetia illucens,_ 10-day-old larvae

Fig. 1: Insect rearing & meal preparation

+10 days

D10

D0

REARING conditions:
- Larvae/substrate proportion: 1/4
- Substrate humidity : ~ 60 %
- Chamber conditions: 24 °C
- Separation by rinsing on sieve
- 24-hour fast (feed restriction)
- Killing: Freezing at - 20°C, 2 days
- Meal preparation:
    - Drying at 40°C, 3 days
    - + grinding

MS-proteomics:

Sample preparation protocol:
- ✓ Extraction: TRIS-urea buffer, pH 9.2
- ✓ Heat treatment: 95 °C, 5 min
- ✓ Denaturation: DTT, IAA
- ✓ In-solution digestion: Trypsin
- ✓ Purification: tC18 SPE (Waters)

UHPLC-MS/MS:
- ✓ Acquity system (Waters)
- ✓ BEH C18 Column; 1.7 µm; 2.1 x 100 mm (Waters)
- ✓ Xevo TQ-XS triple quadrupole (Waters)

Targeted proteins (markers identified in previous studies [1-3]):
- ✓ RUMINANT: Casein, β-lactoglob., haemoglobin & collagen
- ✓ PIG: collagen
- ✓ POULTRY: collagen

[1] M.C. Lecrenier, et al., doi: 10.1016/j.foodchem.2017.11.074.
[2] O. Fumiere, et al., doi: 10.25518/1780-4507.20059.
[3] M.C. Lecrenier, et al., doi.org/10.1021/acs.jafc.3c03253

Results & Discussion

1. Selection of the peptide markers on reference meats

Table 1: Results for UHPLC-MS/MS analyses reference meats

Targeted origin	Proteins	Peptides	Raw meat (Beef)	Cooked meat (Beef)	Beef burger*	Raw meat (Pork)	Cooked meat (Pork)	Ham	Raw meat (Chicken)	Cooked meat (Chicken)	Chicken burger*
Ruminant	Casein	FFVAPFPEVFGK	-	-	5.3E+04	-	-	-	-	-	3.9E+03
	β-lactoglobulin	LSFNPTQLEEQCHI	-	-	-	-	-	-	-	-	-
	Haemoglobin α & β-chain	AAVTAFWGK	1.7E+06	9.3E+05	1.0E+05	-	-	-	-	-	-
		EFTPVLQADFQK	1.1E+06	1.1E+06	1.2E+05	-	-	-	-	-	-
		VGGHAAEYGAEALER	9.4E+05	4.7E+05	1.4E+05	-	-	-	-	-	-
		VVAGVANALAHR	1.7E+07	1.0E+06	9.5E+05	-	-	-	-	-	-
	Collagen I chain α-2	GEPGPAGAVGPAGAVGPR	-	2.3E+05	1.1E+06	-	-	-	-	-	-
		GSTGEIGPAGPGPpGGLR	-	9.1E+04	2.9E+05	-	-	-	-	-	-
		GPpGESGAAGPTGPIGSR	-	4.6E+04	1.7E+05	-	-	-	-	-	-
Pig	Collagen I chain α-2	GFpGSpGNVGPAGK	-	-	-	-	1.1E+05	2.4E+05	-	-	-
		GlpGEFGLpGPAGPR	-	-	-	1.5E+03	5.9E+05	1.4E+06	-	-	-
Poultry	Collagen I chain α-2	GNVGLAGPR	-	-	-	-	-	-	-	1.4E+05	1.8E+05
		GLHGEFGVpGPAGPR	-	-	-	-	-	-	-	1.4E+05	1.9E+05
		GLVGEpGPAGAK	-	-	-	-	-	-	-	1.2E+05	9.2E+04
		GEIGPAGNVGPTGPAGPR	-	-	-	-	-	-	-	6.9E+03	2.0E+04

Mass spec (Peak area)

- **Ruminant haemoglobin peptides:** detected in all beef meats (raw, cooked and industrial burger)
- **Collagen peptides:** detected in their respective meats, except in raw meats
- **Milk proteins:** detected in some industrial products. According to the labelling, these products may contain traces of milk (*).

👍 for cooked & industrial meat

2. Evaluation of substrates and insect meals by MS-proteomics

Substrates:
- **D0:** All meat peptides are detected in respective meats.

  Milk proteins are detected in substrate containing Cheese burger & Pizza.
- **D10:** Meats are still detected (by collagen peptides) but peak area are decreased.

  Ruminant haemoglobin and some ruminant & poultry collagen peptides are no longer detected.

  β-lactoglobulin (milk) is still detected in substrate with Cheese burger & Pizza.

Mass spec (Peak area)

Targeted origin	Proteins	Peptides	Blank Substrate D0	Blank Substrate D10	Blank Insect meal D10	Cheese burger Substrate D0	Cheese burger Substrate D10	Cheese burger Insect meal D10	Pizza Substrate D0	Pizza Substrate D10	Pizza Insect meal D10	Nuggets Substrate D0	Nuggets Substrate D10	Nuggets Insect meal D10
Ruminant	Casein	FFVAPF[...]	-	-	-	1.3E+06	-	-	2.8E+03	-	-	-	-	-
	β-lactoglobulin	LSFNPT[...]	-	-	-	1.4E+04	1.3E+03	-	9.9E+04	1.9E+04	-	-	-	-
	Haemoglobin α & β-chain	AAVTAF[...]	-	-	-	4.1E+03	-	-	-	-	-	-	-	-
		EFTPVL[...]	-	-	-	4.8E+03	-	-	-	-	-	-	-	-
		VGGHAA[...]	-	-	-	4.7E+03	-	-	-	-	-	-	-	-
		VVAGVA[...]	-	-	-	4.8E+04	-	-	-	-	-	-	-	-
	Collagen I α-2 chain	GEPGPA[...]	-	-	-	4.6E+04	-	-	-	-	-	-	-	-
		GSTGEI[...]	-	-	-	1.4E+04	8.0E+03	1.0E+03	-	-	-	-	-	-
		GPpGES[...]	-	-	-	1.3E+04	1.1E+04	1.3E+03	-	-	-	-	-	-
Pig	Collagen I α-2 chain	GFpGSp[...]	-	-	-	-	-	-	5.3E+04	1.7E+04	-	-	-	-
		GlpGEF[...]	-	-	-	-	-	-	1.8E+05	1.8E+04	7.0E+03	-	-	-
Poultry	Collagen I α-2 chain	GNVGLA[...]	-	-	-	-	-	-	-	-	-	2.0E+05	-	-
		GLHGEF[...]	-	-	-	-	-	-	-	-	-	9.2E+04	-	-
		GLVGEp[...]	-	-	-	-	-	-	-	-	-	8.4E+04	3.3E+04	-
		GEIGPA[...]	-	-	-	-	-	-	-	-	-	1.2E+04	-	-

Table 2 : Results of substrates (D1 & D10) & insect meals (D10) analyses

Insect meals:
- **Ruminant & Pig meats** are detected in insect meals produced on Cheeseburgers & Pizza, but poultry peptides are no longer detected in case of nuggets.
- Milk proteins are no longer detected

Fig. 2: Chromatograms of the 2 most intense transitions of ruminant and pig collagen peptides

GSTGEI[...] GPpGES[...]

Insect meal from Subst. + Cheese burger

GFpGSp[...] GlpGEF[...]

Insect meal from Substr.+ Pizza

Conclusion

After the insect rearing process, insects are separated from the feed substrate, but residual feed materials remained stuck on the larvae.
Meat residues were detected in insect meals by MS-proteomics.

Degradation of proteins are not the same for all proteins (haemoglobin _vs_ collagen _vs_ milk proteins). Collagen seems to be a good marker for the monitoring of the use of prohibited products.
The use of MS-proteomics makes possible to distinguish whether the presence of ruminant DNA comes from an authorized (milk) or prohibited ingredient (meat).
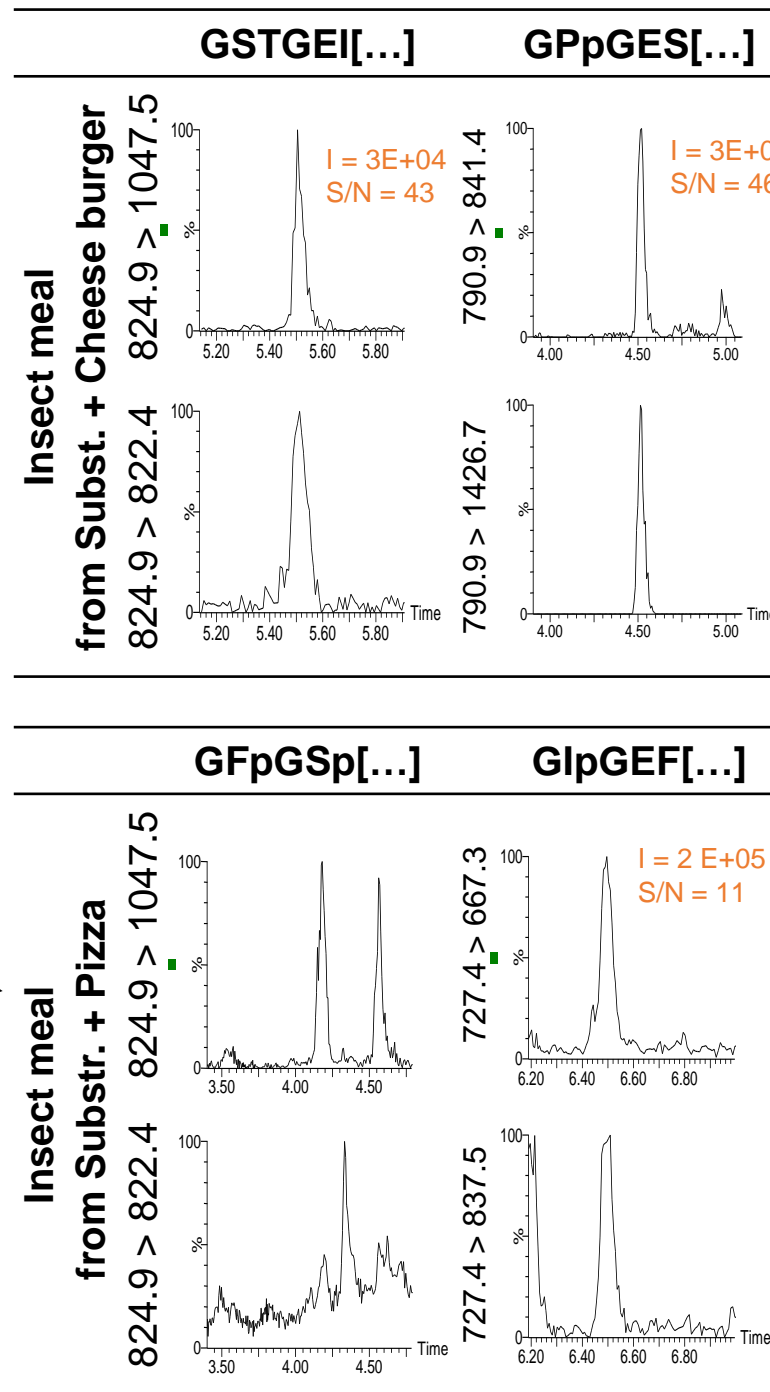
Fig. 3: Feed residues remain stuck on insects

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