high-value products from agricultural residues through sustainable chains

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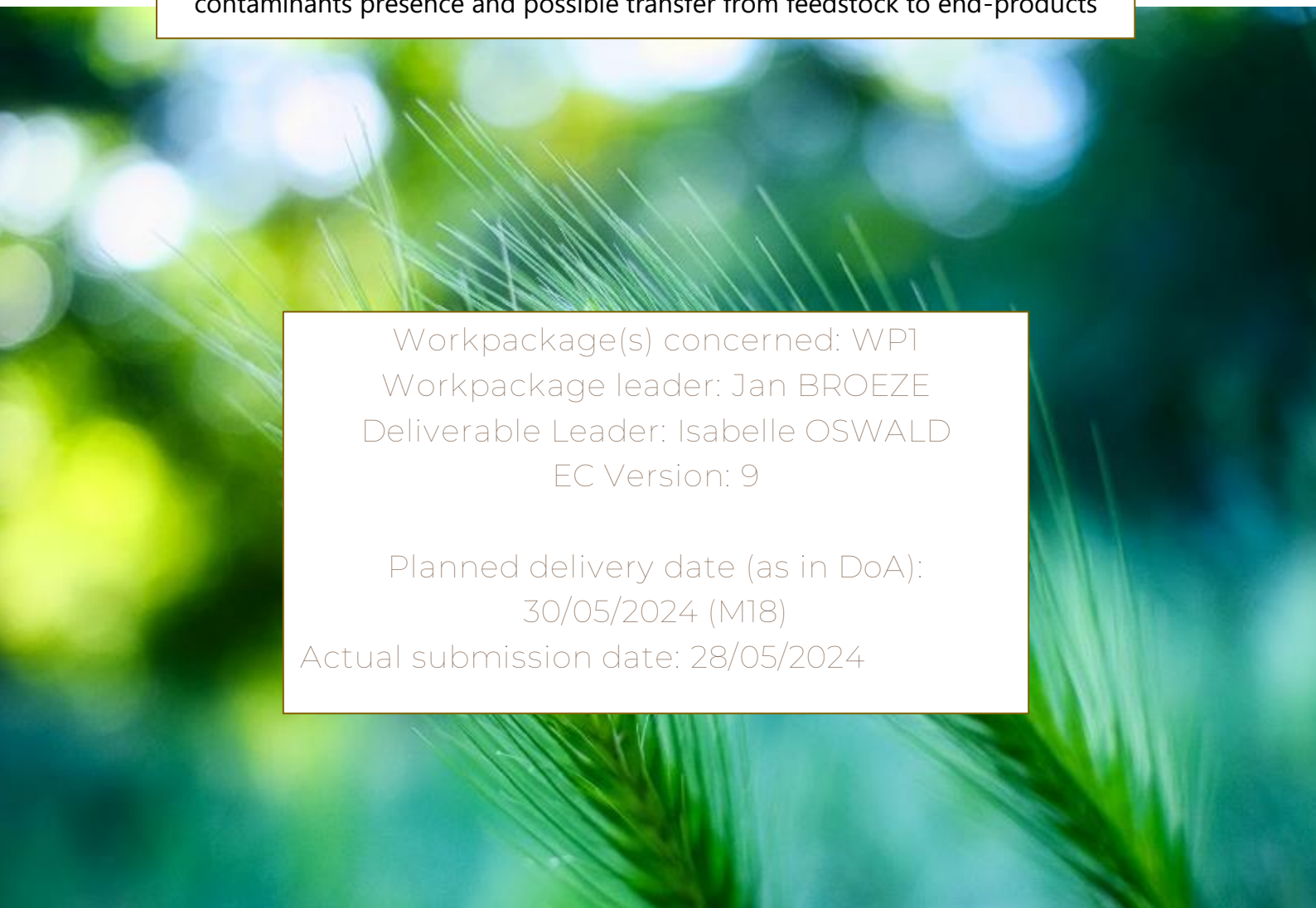
First report on contaminant circular safety: safety aspects related to contaminants presence and possible transfer from feedstock to end-products



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Type

R	Document, Report	☒
DEM	Demonstrator, Pilot, Prototype	☐
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Other	(Please describe the type)	☐

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1. Executive Summary

Safety analysis is crucial to ensure that the derived products issued from the circularized food residues do not contain contaminants potentially hazardous to human or animal health. Indeed, the agri-residues identified in the Agriloop project could be contaminated by natural toxins produced by fungi (mycotoxins), industrial crop protection products (pesticides) or plastic microparticles coming from the environmental or agricultural practices. The purpose of the present work was to assess the level of contamination of several agri-residues used in the project. The main mycotoxins potentially present in the agri-residues were analyze in five samples of raw materials used in the project. Among 61 mycotoxins tested, only six were detected in brewer spent grains (BSG), potato peels and tomato pomace, no mycotoxin could be found in potato peels and tomato grains. The highest level of mycotoxins was found in BSG. An analytical UHPLC–MS/MS protocol for the identification and quantification of the main 13 pesticides contained in these agri-residues has been developed. Five pesticides were detected in tomato peels, potato peels and BSG, but the level remain low. A method to extract and quantify plastic particles has been set up. Image analysis carried out on four types of agri-residues showed systematic contamination by plastic particles. Plastic microparticles were found in tomato peels and tomato pomace in higher amount that in grape pomace and much less were detected in BSG. The black colour of plastic particles suggests a main contamination by the plastic materials used in agricultural environments (buckets, mulching film, etc.).

	AGRI-RESIDUES					
CONTAMINANTS	Tomato grains	Tomato peels	Tomato pomace	Potato peels	BSG	Grape pomace
Mycotoxins						
Zearalenone	< LOD	< LOD	< LOD	< LOD	+	NA
Enniatin A	< LOD	< LOD	< LOD	< LOD	+	NA
Enniatin A1	< LOD	< LOD	< LOD	< LOD	++	NA
Enniatin B	< LOD	+	+	< LOD	+++	NA
Enniatin B1	< LOD	< LOD	< LOD	< LOD	++	NA
Methyl ether alternariol	< LOD	+	< LOD	< LOD	< LOD	NA
Pesticides						
Pirimiphos	NA	< LOD	NA	<LOD	**	NA
Fluxapyroxad	NA	**	NA	***	**	NA
Fluopyram	NA	*	NA	***	***	NA
Tebuconazole	NA	< LOD	NA	**	< LOD	NA
Malathion	NA	< LOD	NA	*	< LOD	NA
Plastic microparticles						
	NA	NA	###	###	#	##

NA: not analysed LOD: limit of detection

+, ++, +++ indicate mycotoxin levels ratio versus LOQ: +(1 < value < 10); ++(10 < value < 20); +++ (value > 20)

*, **, *** indicate pesticides level ratio versus LOQ: *(1 < value < 5); ** (5< value < 10); ***(10< value < 20)

#,##, ### amount of microplastic particles (μm^2/ g material): #(value< 100); ##(100 < value < 1000); ###(value > 3000)

Introduction

Agricultural waste and by-products such as brewery's spent grains, tomato pomace, potato peels, grape pomace or apple peels are a sustainable source for the production of plant and microbial proteins, polyesters and other bio-based chemicals which can be used in the food, feed, pharmacy, materials or cosmetic industries. One of the key challenges of this novel agri-residue bioconversion is to ensure the resulting quality of end-products. Indeed, since the raw materials can be the subject of microbial, chemical or physical contamination in the field or during the storage, it is important to verify the safety of the end-products, especially when they will be destined to food, feed or pharmaceutical applications.

A list of virus, bacteria, protozoans and fungi were identified as potential contaminants (MS9, November 2023), coming from soil, polluted water, handling and storage. However, these living organisms can be destroyed during transformation process. Indeed, numerous physical treatments (heating, ozonation, freezing, UV light, gamma irradiation, high pressure) or chemical treatments (sodium hypochlorite, organic acid, chlorin) are renowned to kill these biological contaminants. However, even when microbiological contaminants are eliminated, the toxins they have already produced can remain in the material so that they represent a real danger for animal or human health. It is the case for mycotoxins produced by molds. Indeed, mycotoxins are secondary metabolites produced by fungal species that can contaminate and grow in the fields but also during transport of on agricultural commodities or processed food. They are organic substances with chemical structures that render them highly resistant to chemical and heat treatment so that they can contaminate food and feed commodities. Furthermore, an agricultural product can be contaminated by several fungi and a fungus can produce various mycotoxins. Thus, co-contamination of products by several mycotoxins is a common situation. A variety of fungal species mostly from genera *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* or *Claviceps* are known to produce mycotoxins. Most important in terms of toxicity and occurrence are aflatoxins B_1, B_2, G_1, and G_2 (AFB_1, AFB_2, AG_1, AFG_2); ochratoxin A (OTA); fumonisins B_1, B_2, and B_3 (FB_1, FB_2, FB_3); deoxynivalenol (DON) and other trichothecenes; zearalenone (ZEN); patulin (PAT); and ergot alkaloids (EAs). Each mycotoxin can have various toxic properties targeting various body organs and therefore represent a danger for human and animal health. Therefore, the dietary or environmental exposures to mycotoxins can trigger various health. Maximum levels for major mycotoxins allowed in food have been established worldwide (EU regulation 2023/915). Good agricultural practices, plant disease management, and adequate storage conditions limit mycotoxin levels in the food chain but do not eliminate mycotoxins completely.

Pesticides play a crucial role in modern agriculture, aiding in the protection of crops from pests, diseases, and weeds. Even if these benefits are present, their use also raises concerns about environmental and human health impacts. When pesticides are applied to crops, residues can remain in the final product or in the soil, posing potential risks to both the environment and human health (1,2). The detection of pesticides' presence in agricultural products and residues is essential for several reasons. Firstly, it ensures compliance with regulatory standards and maximum residue limits (MRLs) set by governing bodies to protect consumer health (3,4). However, according to FAO (Food and Agriculture Organization of the United Nations), at the global level, total pesticides trade reached approximately 5.9 million tonnes in 2018, with a value of $37.6 billion (5). According to Eurostat, in the

period from 2011 to 2018, the consumption of pesticides persisted to be substantially stable at around 360,000 ton/year, remaining concentrated in four main countries (Germany, France, Italy and Spain). In particular, the best-selling chemical formulations were fungicides (45%), herbicides (32%) and insecticides (11%) (6).

In particular, the EU Pesticides Database (https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/start/screen/products/details/116) offers comprehensive data, while the "*What's On My Food*" American website (https://www.whatsonmyfood.org/food.jsp?food=TO) provides valuable insights into pesticide residues in food. However, it is important to note that in the European Union, the allowable levels of pesticide residues in both plant- and animal-derived foods are regulated by the Commission Regulation (EC) No 396/2005. These MRL values are subject to regular update based on the latest toxicological and safety data.

Monitoring pesticides helps to prevent the sale and consumption of contaminated food products, thereby reducing the risk of acute and chronic health effects associated with pesticides exposure. The connection between the presence of pesticides in agro-products and human health is a significant concern. Chronic exposure to pesticides has been associated with various adverse health effects, including neurological disorders, reproductive problems, and certain types of cancer (7–10). This suggests that these biocides may accumulate in the food/feed chain and agroecosystems, leading to long-term health risks for consumers (11,12). Even low levels of pesticides in food can pose health threats over time, especially when individuals are exposed to multiple pesticides through their diet. Overall, adherence to good agricultural practices and regulatory standards can help minimize risks to consumers. By implementing robust monitoring programs and regulatory measures, stakeholders can mitigate the risks associated with pesticides use and promote the sustainable production of safe and nutritious food for present and future generations.

The determination of compounds at trace levels belonging to different chemical classes in complex plant matrices requires sensitive and selective analytical methods. For this purpose, the technique of choice is mass spectrometry preceded by gas or liquid chromatography, with the former most suitable for apolar compounds and the latter most suitable for polar/ionic compounds. In particular, UNIROMA contributed to building an inventory of contaminants, from which the analytical method for their quantification was later implemented. More specifically, a target screening approach was performed by using the ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS) which allows the detection of compounds in a broad range of polarity, except the most apolar and volatile ones.

A great deal of research into plastic pollution has focused on aquatic ecosystems, particularly oceans. In 2020, however, the FAO (Food and Agriculture Organization of the United Nations) warned that agricultural soils could be contaminated by far greater quantities of microplastics. There are many sources and mechanisms of contamination, but two main routes have been identified. (i) The first one is linked to the massive introduction of plastics into farming practices, with the use of plastic mulching film to cover the soil and reduce the presence of weeds, tarpaulins and netting used in the manufacture of greenhouses, sheathing to protect young plants, and plastic containers for pesticides and fertilisers (ii) The second source of contamination is sludge from water treatment plants. The biological treatments applied are unable to break down and eliminate the plastic particles that persist in the

sludge, which is then used for agricultural spreading and composting. Plastic pollution has thus become ubiquitous in agricultural soils, threatening food safety, human health and the environment. Plastic particles can contaminate crops directly, but they can also be incorporated into plants systemically, following absorption through tiny cracks in the root system. Several recent studies have revealed the presence of micro- and nanoplastics in various samples of fruit and vegetables intended for supermarkets or introduced into short distribution circuits. The nature of plastics (or constitutive polymers) varies according to the plant's environment: atmospheric particles are mainly made up of textile fibers but can also come from tyre abrasion or plastic packaging waste. Particles in the soil mainly come from irrigation wastewater treatment plants and animal waste but may also come from degraded mulch film.

In this context, the task 1.3 of the AgriLoop project was focused on the inventory and the analysis of these stable chemical and physical contaminants in raw materials and end products. Indeed, it is crucial to detect these contaminants for ensuring food safety, protecting the environment, and safeguarding human health. **To predict the safety of the end-products, it is of prime importance to understand the effect of the different process on those contaminants: do the various process eliminate these contaminants or lower their level or on the contrary do they concentrate the contaminants in the end-product?**

3- State of the art

3-1 Mycotoxins

Tomato

Tomatoes (*Solanum lycopersicum)* varieties have high carbohydrate and moisture contents, thin skin and are susceptible to fungal contamination. Some fungal contaminants of tomatoes are toxigenic species that belong to genera *Alternaria, Aspergillus, Penicillium* and *Fusariums* (13).

Alternaria spp. (especially *A. alternata*) was reported to be one of the main fungal contaminants of tomato and tomato products (14, 15). *Alternaria spp.* are capable of producing about 30 metabolites with possible toxicity associated with a diversity of potential cocktail effects (16). The toxins of greatest current concern are alternariol (AOH), alternariol monomethyl ether (AME), altertoxins with in vitro mutagenic and genotoxic effects and tenuazonic acid (TeA) with in vitro ribotoxic effects. Among the mycotoxins produced by *Alternaria* species, TeA, AOH and AME are the toxins with the highest prevalence rate and found in the greatest quantities in food products. AOH and AME were shown to be fetotoxic in mouse but the lack of data on their long-term in vivo toxicity prevents regulatory decisions about hazard to human and animal health.

The European Recommendation 2022/553 of April 5, 2022 sets indicative levels for AOH, AME and TeA in a number of foodstuffs, such as processed tomato products. Above these thresholds, the cause of the contamination must be investigated. For processed tomato products, the levels are 10, 5 and 500 µg/kg respectively for AOH, AME and TeA.

Some *Aspergillus spp*. are able to grow on tomatoes (17). *A. niger* has the potential to produce two groups of potentially carcinogenic mycotoxins: fumonisins and ochratoxins (the latter is classified as possible human carcinogen by IARC) (18, 19). *A aculeatus* produces secalonic acid (20, 21) which is

embryocidal and teratogenic as well as fetotoxic when given to female mice during pregnancy. *Aspergillus flavus* is among the most common pathogenic fungi affecting cherry tomato fruits, and it grows over the fruit surface, producing aflatoxin B1 that is a genotoxic, immunotoxic and hepatocarcinogenic metabolite to human beings (classified group 1 by IARC).
Penicillium expansum the causative agent of Blue Mold Disease in apple fruits, has been shown to also infect tomatoes and produce the mycotoxin patulin (PAT) (22, 23). PAT is a regulated mycotoxin showing genotoxic, teratogenic, neurotoxic and immunotoxic properties. PAT is often found as a natural contaminant of apples and their products, but has also been detected in tomatoes (24). Although PAT is considered to be mutagenic, it was included in Group 3 by the International Agency for Research on Cancer (IARC) among those substances "not classifiable as a carcinogen to humans" and is submitted to European regulation.
In humans, *Fusarium oxysporum* is an opportunistic pathogen infecting individual with compromised immunity. Mycotoxicosis in humans and animals following ingestion of food contaminated by toxin-producing Fusarium spp. has been reported a long time ago (Nelson, P.E et al. 1994) (25). The main toxic metabolite produced by *Fusarium oxysporum* when grown on tomatoes is fusaric acid (26, 27) which displays immunosuppressive effects.

Potato

Potato storage conditions, such as relative humidity (80–90%), temperature, long storage time (up to 8 months), and soil contamination are favourable to mould growth. Indeed, potato is susceptible to postharvest rots caused by a variety of fungal pathogens, including *Fusarium spp., Alternaria spp.* and *Rhizoctonia solani* which produce mycotoxins that represent a potential risk for animal and human health (28–31). There are only few studies which have described mycotoxin occurrence in potato plants, they mainly concern the presence of mycotoxins produced by *Fusarium spp.*, especially mycotoxins from the trichotecene family (28,29,30). The *Fusarium* species responsible of the potato dry rot are *F. graminearum, oxysporum, culmurum, equiseti, sulphureum, crookwellense, sambucinum*, and *solani*, the two latters being the most predominant. These fungi can produce a high number of mycotoxins like trichotecenes, fumonisins, enniatins, zearalenone, fusaric acid. Low amounts of these mycotoxins may impair intestinal health and immune function in humans and animals.

Grape

Pathogenic Fungi that commonly infect berries include *Alternaria* spp. *Aspergillus* spp., *Cladosporium* spp., *Penicillium* spp. (32–36). Strains of *Penicillium expansum, Alternaria alternata*, *A. astroemeriae* and *A. fumigatus* isolated from grapes may produce respectively patulin (32), AOH, AME and TeA (14) gliotoxin and verruculogen (36, 37).
However, mycotoxins such as these are seldom detected in wine and other grape products and are currently of little concern for the grape and wine industries (35, 36).
The main mycotoxin of concern in grape and wine is ochratoxin A (OTA) produced by black *Aspergillus spp.* OTA is one of the most potentially dangerous fungal metabolites for human health, with nephrotoxic, hepatotoxic, teratogenic, and carcinogenic effects (38). OTA is produced primarily when *Aspergillus carbonarius* infects berries before harvest. The relatively few toxigenic strains of the related species, *A. niger*, may also contribute to OTA contamination, as *A. niger* is by far the most common species of *Aspergillus* present on grapes (35). A study carried out on Greek wine showed

that 98% of OTA was produced by *A. carbonarius* isolates and only less than 2% for *A. niger* isolates (39). Hot weather favors the development of *A. niger* and *carbonarius, explaining* the increased incidence of OTA in wines from the warmer regions of southern Europe (40).
It was reported that during winemaking 50–80% of the total OTA content spiked in the grapes was bound to the discarded skins and seeds and thus remains in the grape pomace (41, 42). Yeast cell walls, mainly composed of mannoproteins, contribute to further adsorb the toxin and reduce its concentration in the vine. Maximum level of OTA was limited at 2 µg/L in wines and grapes by the European Union (Commission regulation No 123/2005 amending Regulation No 446/2001).
The identification of fungi from grape pomace (43) reported *Aspergillus fumigatus* and *A. niger*, the former producing gliotoxin and a spore born trypacidin, an emerging mycotoxin (44), *fusarium solani* (associated to human keratitis and corneal scraping) and *Penicilium expansum* that produce patulin.

Apple

Penicillium expansum is responsible for blue rot of apples during storage. *P. expansum* can be detected on the surface of apples using real-time PCR. The number of contaminated apples can increase rapidly (15 fold in 24hours) with the time of storage in the harvesting bin (45).
P. expansum is considered as the main source of patulin (PAT) in apple and in its derived products. Considering its toxic effects observed in rodents, PAT was considered to be a risk for human and animal health. Acute exposure results in convulsions, ulcerations, intestinal inflammation, vomiting, edema and DNA damage in the brain, liver and kidneys while chronic exposure leads to neurotoxic, immunotoxic and teratogenic effects (46). Therefore, the acceptable daily intake for PAT was established by the European Commission (EC) and the Joint FAO-WHO Expert Committee on Food Additives at 0.4 µg/kg body weight/day (47). Since the major route of human exposure is mainly through the consumption of apples and its derived products, many countries have set maximum authorized levels for apple juice and products based on apple pieces intended for infants and young children and for baby foods (EC No. 915/2023). The International Agency for Research on Cancer (IARC) has classified PAT in group 3, considered unclassifiable in terms of its carcinogenicity to humans.
Alternaria was found as the main causal agent of mouldy core in Argentina, *A. tenuissima* being the predominant species. Its incidence increased during storage, and most isolates were highly toxigenic, producing AOH, AME, Altertoxin I, Tentoxin and TeA (48, 49). Moreover, its toxins were detected in a high percentage of apple destined for industrialization. Since the disease is not detected by the visual inspection performed by processing industries, contaminated fruit are likely to be incorporated into the process line, with a consequent risk of the presence of *Alternaria* toxins in apple by-products.

Brewer spent grains

However, BSG can support the growth of mycotoxigenic fungi. Indeed, filamentous fungi are frequently isolated after storage of BSG at room temperature, such as *Aspergillus spp., Fusarium spp., Mucor spp., Penicillium spp.*, *Alternaria spp.,* and *Rhizopus spp.* (50). Consequently, trichothecenes, aflatoxins, fumonisins, ochratoxin A, patulin, and zearalenone, are the most significant mycotoxins in the BSG.

The predominant *Fusarium* species in the brewer chain are *F. verticilloides, F. proliferatum , F. equiseti*, *F. culmorum* and *F. oxysporum* (51) which produce the large group of mycotoxins known as the trichotecenes like deoxynivalenol, acetyldeoxynivalenol, nivalenol, zearalenone, fusarenone-X, 3-, diacetoxyscirpenol, T-2, HT-2 toxins. Moreover, fumonisins B1 and AFB1 have been detected in malt barley and BSG from Argentinian breweries but only few traces were found from EU breweries (52, 53). Water-soluble mycotoxins tend to remain in the liquid portion so that the mycotoxins do not have the same fate: it was shown that 80–93% of deoxynivalenol present on the malt grain remained in the final beers and only trace amounts were present in the spent grains. On the contrary, zearalenone and 15-acetyldeoxynivalenol were not detected in the final beers and about 60% and 18% respectively of zearalenone and 15-acetyldeoxynivalenol were found in the spent grains (54, 55).

European regulation

The regulations do not cover all the mycotoxins known to date. Mycotoxins not covered by the regulations are known as emerging mycotoxins. The table 1 below summarizes the minimum and maximum regulated (bold police) or recommended (italic police) mycotoxin levels for food and feed in Europe.

Table 1: mycotoxin regulation in Europe

	Human nutrition[(a)]		Animal nutrition[(b)]	
	Raw materials	Food products	Raw materials	Food products
Aflatoxin B1	**2 - 12 µg/kg**	**0.10- 8 µg/kg**	**20 µg/kg**	**5-20 µg/kg**
Aflatoxins B1+B2+G1+G2	**4-15 µg/kg**	**4-10 µg/kg**	-	-
Aflatoxin M1	-	**0.025 – 0.050 µg/kg**	-	-
Ochratoxin A	**2-20 µg/kg**	**0.5-80 µg/kg**	*250 µg/kg*	*10-100 µg/kg*
Deoxynivalenol	**750-1750 µg/kg**	**200-1250 µg/kg**	*8000-12000 µg/kg*	*900-5000 µg/kg*
Zearalenone	**75-350 µg/kg**	**20-400 µg/kg**	*2000-3000 µg/kg*	*100-500 µg/kg*
Fumonisins B1+B2	**1000-4000 µg/kg**	**200-2000 µg/kg**	*60000 µg/kg*	*5000-50000 µg/kg*
Citrinin	-	**100 µg/kg**	-	-
Patulin	-	**10 – 50 µg/kg**	-	-
Ergot	**0.2-0.5 g/kg**	-	**1 g/kg**	-
T2+HT2 toxins	*50-1000 µg/kg*	*15-100 µg/kg*	*500-2000 µg/kg*	*50- 250 µg/kg*
alternariol	*30 µg/kg*	*2-10 µg/kg*	-	-
Alternariol monomethyl ether	*30 µg/kg*	*2-10 µg/kg*	-	-
Tenuazonic acid	*100-1000 µg/kg*	*500 - 10 000 µg/kg*	-	-

[(a)] : level in the product independently of its water content

[(b)] : water content of 12 %

3.2 Pesticides

Pesticides are commonly applied in food cultivation (such as tomato and potato) to protect the crops from pests, diseases, and weeds. However, due to the inherent toxicity and potential environmental impact of pesticides, stringent regulation and regular monitoring of their residues in food and the environment are required, as stated by the WHO (https://www.who.int/news-room/fact-sheets/detail/pesticide-residues-in-food). In EU, the last report on pesticide residues in food published

in 2024 by EFSA assessed that risk to EU consumer's health is low (56). Based on literature data, this paragraph describes the main types of pesticides possibly present in three types of agro-residues.

Tomato peels

As for tomato cultivation, while pesticides play a crucial role in ensuring high yields and quality products, their residues can remain on various parts of the plant, including the peels of the fruit. The presence of pesticide residues in tomato peels raises concern for potential health risks to consumers. Pesticides can accumulate on the surface of tomatoes during cultivation, especially if frequently and/or improperly applied. Additionally, some pesticides may penetrate the outer layers of the fruit, making it challenging to remove residues through washing or peeling alone. The types and levels of pesticide residues in tomato peels can vary depending on several factors, including the specific pesticides used, application rates, timing of application, and post-harvest handling practices (57). Common pesticides used in tomato cultivation include insecticides, fungicides, and herbicides, each with its own set of potential health and environmental risks. Studies indicate that in processed forms like ketchup and paste, pesticide residues, particularly organochlorine and organophosphorus types, are often undetectable (58). For instance, research by Abou-Arab (1999) (57) found that tomato skins contain elevated levels of pesticides such as hexachlorobenzene (HCB), lindane, dieldrin, and DDT derivatives.

In detail, HCB was commonly used as a fungicide until its ban due to its persistence in the environment and potential health risks. HCB is highly toxic and can bioaccumulate in the food chain, posing risks to human health, particularly through contaminated food consumption (59). Lindane, another organochlorine pesticide, was primarily used to control pests such as lice and scabies in agriculture and medicine. However, its use has declined due to concerns about its toxicity and environmental persistence. Lindane exposure has been linked to various health issues, including neurological disorders and cancer (60). Dieldrin, a persistent organic pollutant, was widely used as an insecticide to control pests in crops like cotton and vegetables. It is highly toxic to both humans and wildlife and has been associated with adverse health effects, including neurotoxicity and carcinogenicity. Dieldrin is known to bioaccumulate in the environment and can persist for long periods, posing risks to ecosystems and human health (61). DDT was extensively used as an insecticide in agriculture and for vector control to combat diseases such as malaria and typhus. Despite its effectiveness in controlling insects, DDT's persistence in the environment and adverse effects on wildlife and human health led to its ban or severe restrictions in many countries. DDT derivatives, which include breakdown products of DDT, can also pose health and environmental risks due to their persistence and potential for bioaccumulation (61,62).

Overall, the use of these organochlorine pesticides has declined significantly due to their harmful effects on human health and the environment. However, their persistence in the environment means that residues may still be found in certain food products, agro-residues, and ecosystems, highlighting the importance of continued monitoring and regulation to minimize exposure and protect public health and the environment.

However, practices like washing, freezing, and peeling have been shown to reduce pesticide residue levels. Additionally, the storage of tomatoes can influence pesticide concentrations during the pre-

harvest interval. Research by Moura et al. (2020) (63) revealed that tomatoes stored at lower temperatures during this period exhibited less reduction in pesticide levels compared to those left in agricultural fields. Thus, adopting appropriate harvesting methods is essential to minimize pesticide residues and protect consumer health. Moreover, cooking tomatoes has been identified as an effective way to reduce pesticide residues, offering an additional layer of protection against potential health risks associated with pesticide exposure (57).

Potato peels

The types of pesticides used in potato cultivation can vary, as insecticides that are employed to control pests such as Colorado potato beetles, aphids, and potato tuber moths. While fungicides help prevent fungal diseases like late blight, early blight, and powdery mildew, herbicides are used to manage weeds that compete with potato plants for nutrients and water (2,62). Pesticide residues can be present on the surface of potato peels as a result of direct application to the foliage or soil, as well as through systemic uptake by the plant. Residues may also accumulate in the peel due to their hydrophobic properties, which can cause them to adhere to waxy surfaces. Also in this case, through washing and peeling of potatoes can help reduce exposure to residues on the surface. Additionally, cooking potatoes at high temperatures can further minimize pesticide residues, as many pesticides break down or degrade when exposed to heat (64).

However, several literature studies have examined the presence of pesticides in potato residues. For instance, in a study by Zohair (2001) (65), higher levels of organophosphorus compounds such as pirimphos methyl, malathion, and profenofos were found. It is worth noting the political context as well, for example in Egypt where the complete cessation of heptachlor epoxide and DDT under the Pest Control Program by the Ministry of Agriculture was imposed (65). Nevertheless, organochlorine compounds like lindane, aldrin, heptachlor epoxide, and DDT derivatives in potato samples obtained from the local market in Egypt are still found. This underscores how contamination by these pesticides likely occurred before their ban, suggesting their penetration into the samples and stability over time.

In contrast, a study by López-Pérez et al. (2006) (66) found that the only pesticide detected in potato tubers was metalaxyl, which was also the sole pesticide detected in wash-off due to its higher solubility in water compared to other pesticides used on the growing plants. Metalaxyl is a systemic fungicide utilized to combat fungal diseases in crops. It effectively inhibits the growth of fungi, particularly those belonging to the oomycete family, which are responsible for diseases such as late blight in potatoes and downy mildew in other crops (67). Applied to soil or foliage, metalaxyl is absorbed by plant tissues, offering protection against fungal infections. While metalaxyl is considered relatively safe for mammals, prolonged exposure can pose risks to human health and the environment (68).

Furthermore, research by Garron et al. (2009) (68) investigated the impact of certain pesticides sprayed in the air on potato fields, such as carbofuran and diquat dibromide and paraquat. Regarding carbofuran, it is a broad-spectrum carbamate insecticide and nematicide employed to control insects in various crops. This compound disrupts the nervous system of insects and nematodes, providing rapid control of target insects (69). However, its toxicity extends to non-target organisms like birds, mammals, fish, and beneficial insects, leading to regulatory restrictions in many countries. Despite its

efficacy, the environmental persistence of carbofuran raises concern about its long-term impact on ecosystems (8). Diquat and paraquat are non-selective herbicides widely used to manage weeds in agricultural and non-agricultural settings. Applied as a foliar spray, they quickly desiccate and kill plants by disrupting photosynthesis and causing cellular damage (65,70). However, even if diquat breaks down relatively quickly in the environment, it can still pose risks to aquatic ecosystems if it enters water bodies through runoff or spray drift.

Overall, these studies shed light on the complex dynamics of pesticide residues in potatoes, highlighting the influence of various factors such as pesticide type, application methods, environmental conditions, and regulatory measures.

Brewery Spent Grain

Brewery spent grain, a by-product of the brewing industry, is primarily composed of the residues of malted barley and other grains after the mashing process (71). While the brewing process itself does not involve the direct application of pesticides, residues from pesticide-treated crops used in malting barley cultivation can carry over into the spent grain.

The potential for pesticide residues in brewery spent grain depends on various factors, including the farming practices employed, the types of pesticides used, and the extent of pesticide degradation during malting and brewing processes (72). Pesticides commonly applied in barley cultivation include azoxystrobin, bixafen, fluopyram, fluxapyroxad, mandipropamid, metrafenone, piperonyl butoxide, and tebuconazole that exhibited high occurrence rates (>60%) in brewery spent grain, indicating potential extensive use of these pesticides on food and feed crops (72). In particular, the fungicide azoxystrobin belongs to the strobilurin class of fungicides and works by inhibiting mitochondrial respiration in fungal cells, leading to their death (73). Azoxystrobin is effective against various fungal pathogens, including powdery mildew, rusts, and leaf spot diseases. Bixafen and fluxapyroxad are a broad-spectrum fungicide belonging to the succinate dehydrogenase inhibitor (SDHI) class of fungicides and work by disrupting the energy production process in fungal cells, providing preventive and curative control of a wide range of fungal pathogens, including *Fusarium* spp., *Rhizoctonia* spp., and *Botrytis* spp (74). Mandipropamid is a systemic fungicide belonging to the carboxylic acid amide (CAA) class of fungicides, and works by inhibiting fungal cell growth and development, effective against pathogens such as *Plasmopara viticola* (75). Metrafenone, belonging to ketone class, provides preventive and curative control of diseases caused by pathogens such as *Uncinula necator* and *Podosphaera* spp (76). Piperonyl butoxide (PBO) is a synergist commonly used in combination with insecticides and fungicides to enhance their effectiveness. It works by inhibiting the activity of enzymes that metabolize pesticides in insects and fungi, thereby increasing the potency and duration of pesticide activity. Piperonyl butoxide is often used in formulations with other active ingredients to improve pest control in various agricultural and household applications (77). One potential solution to address this issue involves reducing pesticide levels during the malting and brewing processes, thereby mitigating the risk of contaminating beer with pesticides (78,79). Additionally, research has shown that some pesticides undergo degradation or transformation during boiling and fermentation stages (71).

I- *Analysis of mycotoxins in agri-residues*

I.1. Experimental protocol

The agri-residues used in the Agriloop project could be contaminated by numerous fungi. These fungi can produce mycotoxins, some of them being potentially hazardous for human or animal health. A high number of mycotoxins was analyzed in the five raw materials used in the WP2 and 3:

- Frozen samples of tomato pomace, tomato grains and tomato peels supplied by Tomato Paint (Italy) in February 2023,
- a dried sample of Brewer Spent Grains (BSG) from a local Belgium brewery sent by UNIROMA on December 2023,
- frozen potato peels (Impetus, Belgium) provided by University of Montpellier in November 2023.

The main mycotoxins possibly contaminating these raw materials was identified in a detailed bibliographic study (milestone MS9):

- Tomatoes: Alternaria mycotoxins (alternariol, alternariol monomethyl ether, tenuazonic acid, tentoxin, altenuene), fumonisins, patulin, secalonic acid, fusaric acid,
- Potatoes: trichotecenes family, enniatins, beauvericin, zearalenone, fusarin C, alternariol monomethyl ether, tentoxin,
- Brewer Spent Grains: trichotecenes family, enniatins, zearalenone, patulin, fumonisin, fusarenon, aflatoxins,
- Apple: Alternaria mycotoxins (alternariol, alternariol monomethyl ether, tenuazonic acid, tentoxin, altenuene),
- Grape pomace: Ochratoxin A, alternariol, alternariol monomethyl ether, tenuazonic acid, verruculogen, gliotoxin.

We undertook to analyze the level of these mycotoxins, as well as others mycotoxins not enclosed in the main list, from five samples of raw materials used in the project.

Analytical method

Stock solutions of standard mycotoxins prepared in acetonitrile were solubilized with 0.01% of acetic acid for HPLC-MS/MS calibration. The HPLC was performed using a Hewlett Packard type 1100 (Hewlett Packard, Eybens 38, France) equipped with a Column 250 mm × 4.6 packed with C_{18} phase (VWR Pessac 33, France Applied Biosystems, Foster City, CA, USA). The mobile phase was made up of ammonium acetate 1nM, 0.0001% acetic acid/methanol and 1% acetonitrile. A linear gradient was applied for 40 min at a flow rate of 1 mL/min. Detection was performed with a quadrupole tandem mass spectrometer API 4000 (Applied Biosystems, Foster City, CA, USA) at a source temperature of 500 °C with a 4500 V ion spray voltage in positive and negative mode interface. Each mycotoxin was identified and quantified on two or three transitions. Samples were ground to a fine powder and sifted through a 0.5 mm particle size filter. Five grams of sieved samples were extracted for 2 h by reversal agitation with 20 mL of acetonitrile/water. The extract was centrifuged, and 3 mL of the aqueous phase were evaporated to dryness. The dry residue was dissolved in a solution of 0.01% acetic acid/methanol

(2/1, *v*/*v*), filtered on a syringe, and injected in the HPLC-MS/MS. The limit of quantitation ranged from 1 to 10 µg/kg, depending on the mycotoxin.

II.2. Results

It is worthy to note that the results are not representative of a common situation of contamination, since the diversity and level of mycotoxins depend on growing, transport and storage conditions. The agri-residues were analyzed for their content in 61 mycotoxins. Results (see table in annex) showed that tomato grains and potato peels did not contain levels of mycotoxins exceeding the limit of detection. Some mycotoxins were detected in tomato pomace, tomato peels and brewer's spent grains (Table 2). Both tomato pomace and tomato peels displayed low level of enniatin B produced by *fusarium*, but tomato peels contained also alternariol monomethyl ether (AME: 35µg/kg) from *Alternaria* species. Due to the lack of toxicological data, these mycotoxins are not the subject of regulation in Europe but a Threshold of Toxicological Concern has been applied based on the current scientific knowledge (14). They are 1500 ng/kg b.w/day for enniatins and a lower level for AME, 2,5 ng/kg b.w/day, due to its genotoxic properties. Furthermore, a recommendation n° 2022/553 published by the European commission in 2022 fixed an indicated level at 5 µg/kg for alternariol monomethyl ether in tomato-based products.

BSG contained the most significant level of mycotoxins (both of them produced by *fusarium* species) of the five samples: mycotoxins from the enniatin family (total 563 µg/kg) and a lower level of zearalenone (14 µg/kg) that is under the maximum level in the food indicated in the European regulation (20 to 400 µg/kg in food commodities).

Table 2: analysis of mycotoxins

	µg/kg raw material	µg/kg dry weight			
Mycotoxins	**Potato peels**	**Tomato grains**	**Tomato pomace**	**Tomato peels**	**Brewer Spent Grains**
Zearalenone	**<10**	**<30**	**<30**	**<50**	**14**
Enniatin A	**<5**	**<15**	**<15**	**<25**	**8**
Enniatin A1	**<5**	**<15**	**<15**	**<25**	**55**
Enniatin B	**<5**	**<15**	**9**	**25**	**320**
Enniatin B1	**<5**	**<15**	**<15**	**<25**	**180**
Methyl Ether Alternariol	**<5**	**<15**	**<15**	**35**	**<5**

I.3 Conclusion

The analysis of the food by-products (tomato pomace, tomato peels, tomato grains, potato peels and brewer spent grains) demonstrated that few (six) mycotoxins were present in these raw materials. These mycotoxins were identified as potential contaminants in the bibliographic study previously submitted (Milestone MS9, November 2023). Brewer's spent grains is the agri-residue containing most of them (five/six) and contains the highest level of the enniatins, emerging mycotoxins produced by *Fusarium* species. It is crucial to be able to understand the fate of contaminants through the end

products manufacturing process. In that view the initial content of mycotoxins must be significantly over the LOQ. Taking that in account, only enniatin A1, B, B1 and alternariol monomethyl ether could be measured all along the process from brewer's spent grains and tomato peels respectively. However, in these samples it should still be necessary to spike additional mycotoxins. In that view, the spiked mycotoxins should be chosen because of i) their potential contamination of the agri-residues, ii) their possible different behavior all along the process. Obviously, the other samples (potato peels, tomato grains and tomato pomace) that contain low or no mycotoxin at all should also be spiked with two or three mycotoxins. In that view, the proposed mycotoxins to be spiked are listed below:

- Potato peels: zearalenone, alternariol monomethyl ether and enniatin B
- Tomato grains and pomace: alternariol monomethyl ether, tenuazonic acid and enniatin B
- Tomato peels: tenuazonic acid and enniatin B (alternariol monomethyl ether already present)
- Brewer's spent grains: zearalenone, HT2 toxin (enniatin B already present)

II- *Analysis of pesticides in agri-residues*

II.1. Experimental protocol

For the determination of pesticides content, which are often present at very low concentrations, in complex plant matrices that can interfere with their analysis, the selection of a suitable pre-treatment method is particularly important (80). The Quick, Cheap, Effective, Rugged, and Safe sample preparation method (QuEChERS) is the most commonly used technique for isolating trace contaminants in complex food and plant samples (81-84). QuEChERS enables the minimization of both sample amount and the use of toxic solvents, making it a highly efficient approach.

For this reason, for extracting pesticide residues from the three matrices of interest in WP2 and WP3, namely tomato peels (TP provided to UNIROMA by Tomato Paint srl), potato peels (PP, provided by UGENT), and brew spent grain (BSG, provided by UGENT), a QuEChERS protocol was employed. The protocol consisted of a solvent extraction with acetonitrile and salts, followed by a dispersive solid-phase extraction (dSPE) clean-up step. Then, analysis was performed by reversed phase UHPLC-MS/MS coupled via an electrospray source, using a triple quadrupole mass analyzer in multiple reaction monitoring (MRM) acquisition mode. Recovery experiments of 13 main pesticides (i.e., commonly detected) for the selected crops were carried out to assess the extraction efficiency of the proposed method, with the aim to optimize the analytical condition suitable for all the matrices. Further pesticides could be included in the analysis.

The analytical procedure consisted of the following steps: extraction, clean-up, Instrumental analysis; which are described below. Analyses were carried out in triplicate.

EXTRACTION: 0.5 g of the freeze-dried and previously homogenized sample were placed into a 50 mL centrifuge tube. Then, 5 mL of water were added to rehydrate the matrix. After 15 min, 5 mL of a solution containing 0.5% (*v/v*) formic acid in acetonitrile were added and the mixture was shaken for 10 min. Afterward, 1 g of sodium chloride and 4 g of magnesium sulphate were added, the tube was shaken for 1 minute before being centrifuged for 5 min at 8000 rpm. Finally, approximately 4-5 mL of the supernatant were collected.

CLEAN-UP: A 2 mL aliquot of the extract was placed in a 15 mL centrifuge tube containing Supel™ QuE Z-Sep+ (product number 55486-U by Merck). The mixture was shaken for 1 min, then centrifuged for 2 min at 3300 rpm and finally 1 mL of the extract (cleaned-up extract) was evaporated to approximately 0.1 mL using a speedvac system. The residue was reconstituted to a final volume of 300 µL of a solution consisting of 70/30 (*v/v*) water/acetonitrile, with 100 ng of the internal standard (triphenyl phosphate, TPP). The solution was filtered using a GHP membrane with a pore size of 0.2 µm. Finally, 20 µL were injected into the UHPLC-MS/MS system.

INSTRUMENTAL ANALYSIS: Samples were analyzed by RP chromatography on an Ultimate 3000 LC system (Thermo Fisher Scientific, Bremen, Germany) coupled to a TSQ Vantage triple-stage quadrupole mass spectrometer (Thermo Fisher Scientific) via a heated electrospray interface (ESI) for MRM detection of the target compounds. The UHPLC system consisted of a binary pump equipped with a degasser, a thermostated microwell plate autosampler, set at 14 °C, and a thermostated column oven. Samples were injected onto a Hypersil GOLD™ VANQUISH™ C18 (50 x 2.1 mm i.d., 1.9 µm particle size) equipped with a Security guard C18 (2.1 mm i.d.). The column was thermostated at 50 °C and the mobile phase consisted of (A) H_2O with 0.1% (*v/v*) HCOOH, and (B) MeOH with 0.1% (*v/v*) HCOOH; the flow rate was set at 0.3 mL/min. After a 3 min-isocratic step at 5 %B, B was increased to 60 % in 2 min. Subsequently, within 1 min, B was further increased to 70 %. After the gradient, B was brought to 100% in 4 min and kept constant for 5 min to rinse the column; finally, B was brought back to 5 % and the column was equilibrated for 4 min. Analyte retention time and MRM transitions for the 13 compounds analysed are reported in Table 3.

Table 3. Instrumental acquisition parameters. Product ion (m/z) represents the mass to charge ratio of the monitored fragment ions and CE represents the collision energy set in the mass spectrometric method to obtain them

Compound	RT (min)	Ion Polarity	Precursor ion (*m/z*)	Product ion (*m/z*). Value in brackets represent the CE (V)
Thiamethoxam[a]	5.5	+	292	211 (12), 181 (22), 132 (23)
Imidacloprid[a]	5.9	+	256	209.1 (17), 175.1 (18), 84.3 (19)
Atrazine[b]	7.1	+	216	174.1 (17), 104.1 (28), 68.3 (35)
Malathion[a]	7.6	+	331	99.1 (23), 127.1 (13), 125 (29)
Fluxapyroxad[c]	7.7	+	382	342.1 (23), 362.1 (13), 314.1 (24)
Myclobutanil[c]	7.8	+	289	70.3 (17), 125.1 (32), 151 (25)
Fluopyram[c]	7.9	+	397	207.9 (24), 173 (31), 145 (50)
Diflubenzuron[a]	8.0	-	309	156 (13), 93.1 (47), 42.3 (16)
Fipronyl[a]	8.1	-	436	330.9 (18), 250.8 (29), 318.9 (26)

Pirimiphos[a]	8.2	+	306	108.2 (33), 164.1 (22). 67.3 (37)
Tebuconazole[c]	8.2	+	308	70.3 (21), 125.1 (34), 151 (25)
Pyraclostrobin[c]	8.3	+	388	163 (23), 194 (12), 149 (29)
Trifloxystrobin[c]	8.7	+	409	186 (21), 145.1 (40), 206.1 (14)

[a] insecticide
[b] herbicide
[c] fungicide

II.2. Results

The analytical method was optimized considering the MRLs set by EU for the considered matrices (see Table 4).

Table 4. EU Maximum residue limit (MRL) in mg/kg (source: https://food.ec.europa.eu/plants/pesticides/eu-pesticides-database_en)

Compound	Tomatoes	Potatoes	Barley
Diflubenzuron	0.01	0.01	0.01
Fipronil	0.005	0.005	0.005
Atrazine	0.05	0.05	0.05
Imidacloprid	0.3	0.01	0.01
Myclobutanil	0.6	0.06	0.01
Thiamethoxam	0.2	0.07	0.4
Pirimiphos	0.01	0.01	5.0
Tebuconazole	0.9	0.02	2.0
Malathion	0.02	0.02	8.0
Fluxapyroxad	0.6	0.3	3.0
Pyraclostrobin	0.3	0.02	1.0
Fluopyram	0.5	0.08	0.2
Trifloxystrobin	0.7	0.02	0.5

Recoveries were determined by spiking the three matrices with a mixture of pesticides at different concentration levels, namely between 5-30 µg/kg. Recoveries expressed as range values are reported in Table 5 for TP, PP and BSG.

Table 5. Recoveries for the three matrices (spiking levels in the range 5-30 µg/kg)

Compound	TP	PP	BSG
Diflubenzuron	70-80%	98-106%	87-90%
Fipronil	72-75%	87-99%	96-106%
Atrazine	80-97%	94-99%	81-88%
Imidacloprid	75-112%	85-90%	88-96%
Myclobutanil	72-78%	75-86%	77-85%
Thiamethoxam	78-82%	85-102%	95-107%
Pirimiphos	73-100%	89-110%	112-122%
Tebuconazole	70-96%	75-92%	98-108%
Malathion	71-87%	71-97%	97-107%
Fluxapyroxad	83-102%	76-102%	77-91%
Pyraclostrobin	70-94%	84-106%	77-104%
Fluopyram	98-107%	74-98%	71-112%
Trifloxystrobin	75-80%	70-83%	90-92%

For all the three matrices, matrix-matched calibration was carried out for correction of the signal suppression (electrospray matrix effect). Method limits of detection (LODs) and quantification (LOQs) were determined as the lowest point of the matrix-matched calibration graph with a signal-to noise ratio S/N>3 and >10, respectively (Table 6).

Table 6. Method limits of detection (LODs) and quantification (LOQs).

	TP		BSG		PP	
Compound	LOD (mg/kg)	LOQ (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)	LOD (mg/kg)	LOQ (mg/kg)
Diflubenzuron	0.0015	0.003	0.0024	0.003	0.0011	0.003
Fipronil	0.003	0.030	0.0020	0.003	0.0018	0.003
Atrazine	0.0005	0.003	0.0007	0.003	0.0004	0.003
Imidacloprid	0.0008	0.003	0.0005	0.003	0.0004	0.003
Myclobutanil	0.0029	0.030	0.0031	0.030	0.0028	0.030
Thiamethoxam	0.0006	0.003	0.0004	0.003	0.0004	0.003
Pirimiphos	0.0008	0.003	0.0003	0.003	0.0003	0.003
Tebuconazole	0.0004	0.003	0.0004	0.003	0.0003	0.003
Malathion	0.0006	0.003	0.0028	0.030	0.0007	0.030
Fluxapyroxad	0.0004	0.003	0.0008	0.003	0.0005	0.003
Pyraclostrobin	0.0031	0.030	0.0029	0.030	0.0024	0.030
Fluopyram	0.0007	0.003	0.0002	0.003	0.0001	0.003
Trifloxystrobin	0.0027	0.030	0.0019	0.003	0.0026	0.003

In the analyzed samples, only three pesticide residues were detected in TP and BSG and four in PP (Table 7), all below their MRLs except for tebuconazole and malathion in PP, which were slightly higher than the MRL values. However, it should be noted that MRLs are referred to the entire potato vegetable and not just to the peels (which could be more concentrated in contaminants), as the examined samples.

Based on the obtained data, in order to evaluate the possible transfer of pesticides from the agricultural residues to the end products (e.g., biopolymers), acidogenic fermentation (AF) tests will be performed on the residues (in particular potato peels) implemented with selected pesticides to evaluate the fate of contaminants during the biological AF process.

Table 7. Sample contamination (mg/kg).

Compound	TP	BSG	PP
Pirimiphos	< LOD	0.027	< LOD
Fluxapyroxad	0.021	0.026	0.052
Fluopyram	0.003	0.032	0.053
Tebuconazole	< LOD	< LOD	0.024
Malathion	< LOD	< LOD	0.029

II.3 Conclusion

The quantification of pesticides concentration in agricultural matrices and products is pivotal to ensuring food safety, compliance with regulatory standards, and minimizing adverse health effects associated with chronic exposure. In this context, UNIROMA contributed to the establishment of an inventory of contaminants through a specific literature search on pesticides contained in three selected agricultural matrices (i.e., tomato peels, potato peels, and brewery spent grains). Based on literature data, an analytical protocol for the identification and quantification of the main pesticides contained in the selected matrices has been developed. In particular, many multi-residue pesticide methods have been reported in the scientific literature for fruits and vegetables. However, few methods have specifically addressed peels of agri-food products, which may pose a challenge when evaluating compliance of MRLs in such products.

The performed activity consisted in the analysis of the UHPLC–MS/MS analysis of 13 pesticides typically contained in the three selected agricultural matrices, as retrieved form the literature and monitoring programs data. Following a sample treatment based on the QuEChERS method, with acetonitrile employed as the extracting solvent, an in-depth investigation was conducted on the matrix effects linked to the employment of the ESI source in the UHPLC-MS/MS analysis. The method was validated through linearity, matrix effect, accuracy and precision, LOQs and LODs on the selected matrices. The results showed that the developed method fulfilled the requirements for pesticide residue analysis. The developed method could be used for the routine monitoring of other pesticide residues in the matrices of interest.

III. *Analysis of plastic particles in agri-residues*

Developing a method for extracting and quantifying microplastics raises three major issues. The first concerned the prevention of potential contamination of samples by microplastics from various sources (air, reagents, glassware, etc.) throughout the microplastics extraction protocol. The second related to obtaining digestates (digested solutions) that could be filtered on 0.2 µm alumina filters and that could

be analyzed. Finally, the third challenge is to identify a viable and reliable analysis method for quantifying the number and the compositional nature of microplastics.

III.1. Digestion protocol

From a practical point of view, the particular composition of the residues (rich in lignocellulose and waxy matter) requires specific experimental procedure to ensure prior digestion of the organic matter required for quantitative analysis of the contaminating micro-plastics. The analyses prior digestion was conducted on four agri-residues *i.e*. potato peels, tomato pomace, grape pomace and brewer's spent grain.

The optimum conditions of digestion for the different matrix were preliminary determined on the basis of literature data extracted from the literature (Table 8), with particular attention paid to studies of agricultural products of similar composition.

Table 8: Matrix/digestion affinity

	Oxydative digestion	**Acid digestion**	**Alkaline digestion**
Efficient digestion	Organic matter (present in soil, sludge, wastewater and sediment) (85) Plant matter: especially with fenton's reagent (86) Fatty material (87) Cellulose (87) Chitin shells (87) Bivalves (88) Fishs (88)	Biota (85) Biological tissues (87) Biotic tissues (carbohydrates, proteins, fats) (especially HNO3) (87) Very calcareous environments (HCl 10% at Tmax: 20°C) (87, 89)	Biological tissues (soft and hard) (soft: mussels, oysters, clams) (87,90). Biota (85,87) Plant tissues (91) Animal tissues (86) Bottom sludge (with NaOH +++) (85)
materials resistant to the digestion medium	Calcareous materials (89)	Biogenic matter in sediments (deep-sea carbonates and siliceous deposits) (87)	Sediment (89) Cellulosic and chitinous materials (cellulose, hemicellulose, lignin, plant residues, etc.) (87,89)

Among the large panel of methods tested, the most effective digestion method proved to be oxidative digestion of the Fenton type including hydrogen peroxide (H_2O_2 30%) and an iron (II) solution ($FeSO_4/7H_2O$) (92). The medium must be acidic to use these reagents and allow the $FeSO_4.7H_2O$ salts to dissolve properly, with an optimum pH of 3, which must be less than 5. Digestion is carried out in

the presence of sulphuric acid (H_2SO_4) (85). It was additionally observed that microplastics degrade when the digestion temperature exceeds 50°C and with certain specific reagents (Table 9).

Table 9: Degradation conditions of microplastics in digestive environments

	Oxydative digestion	**Acid digestion**	**Alkaline digestion**
Type of microplastics (MPs) degraded	H_2O_2 at 70°C: PMMA (85) H_2O_2 between 70 and 100°C: PA (87) 30% H_2O_2 at 70°C: PA-6,6, cracking PS (85) Fenton reagent (H_2O_2 + Fe^{2+}): Rubber (93) HCl (20%) + Fenton reagent (H_2O_2 + Fe^{2+}): Chemical structure modification of PA (94)	HNO_3: Degradation of PA, PS and nylon (87) HNO_3: Color change of PE, PP and PVC (87) HNO_3 (15,8 M): PS (88) HNO_3 20 or 65% and HCl 10% or 37%: PA, PET and PS microplastics degradation (89)	NaOH 10 70°C: PET and PC (85) NaOH 1M: « peeling » surface PET and matt surface of PC (85) KOH 10% and NaOH 20%: fabric fibers (85) NaOH and KOH: effects on PET and PC (89) Effects on rubber (93)
Anti-degradation solution	Fenton reagent: less mass loss than digestion with H_2O_2 (85)	Aqua regia= HNO_3: HCl 1: 1 (87)	KOH 10% at 60°C (85) 30% solution KOH: NaClO 1: 1 (87)

For the study presented in this document, oxidative digestion conditions with Fenton's reagent were used, with the digestion temperature set at 50°C in a bath with agitation. Regarding the exothermic potential during the reaction between the two reagents, digestion medium is placed in an ice bath beforehand to prevent/limit potential thermal runaway, then the temperature will be set at a maximum of 50°C for the digestion (85,88).

III.2. Analytical method

Analysis of the contamination of agricultural residue fractions is a multi-step process involving i) grinding specific to each fraction, ii) digestion of the organic matter and iii) analysis of the post-digestion particulate residue. The process developed for each fraction is summarised in the figure 1.

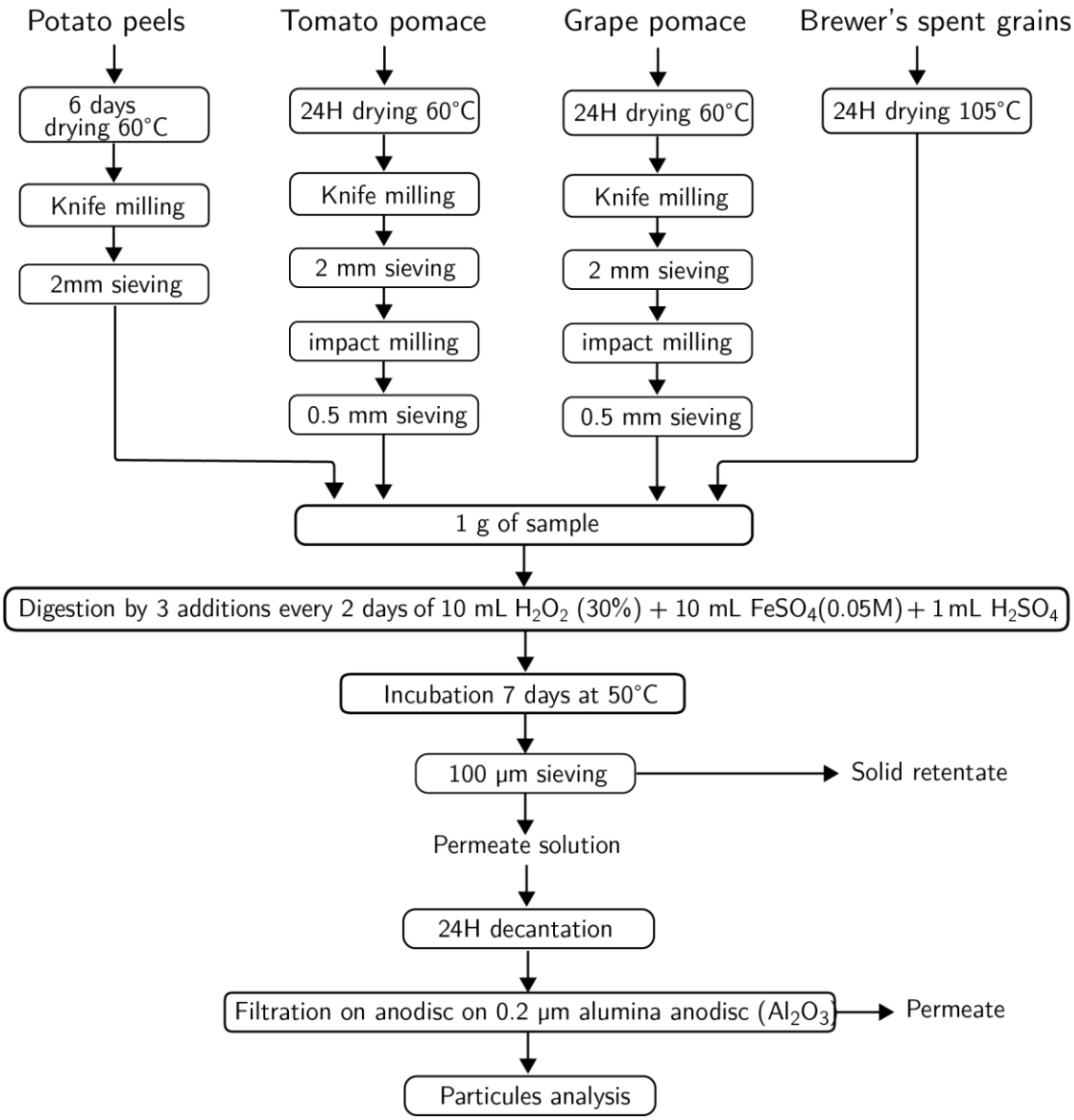


Figure 1: Experimental approach applied to the analysis of the contamination of agricultural residues by plastic particles

III.2.1 Agro-residues

The residues analysed (potato peels, tomato pomace and brewer's spent grain (BSG) come from the same batches as the samples subjected to analysis for contamination by mycotoxins and pesticides. Sample of grape pomace was provided by the company Grap' Sud

III.2.2 Grinding of samples

2.2.1 Retsch SM300 knife grinder

The SM300 shredder uses a cutting and shearing action. The charged material is intercepted by the parallel-section rotor and then shredded between it and the double counter-knives located in the

shredding chamber. It features a 3kW motor with the option of selecting the rotation speed from a range of 700 to 3,000 rpm. This equipment can grind to different dry matter granulometries (water content < 10%): 20 mm, 10 mm, 6 mm, 4 mm, 2 mm, 1 mm, 0.5 mm and 0.25 mm.

2.2.2 AlpineTM UPZ100 grinding station

The UPZ grinding station can be fitted with several different types of mill (one for each application) to reduce powders to ultra-fine particles. The grinding system can be fitted with a pin mill, paddle mill or shear mill. The paddle shredder consists of a mobile shaft made up of paddles that throws the product against a fixed grid. The product remains in the grinding chamber until it is ground. It undergoes both impact and shear crushing (it is thrown against the grate and circulates against it until it has passed through). It is recommended for fibrous products such as bran. However, it is less effective for grinding powders such as flour or semolina. This equipment can grind to different dry matter granulometries (water content < 10%): 4 mm, 3.5 mm, 2 mm, 1 mm, 0.5 mm, 0.3 mm, 0.2 mm and 0.1 mm.

III.2.3 Water content measurements

Water content measurements were carried out using a PRECISATM XM60 desiccator. This enables the water content in liquid, porous or solid materials to be measured quickly. This measurement is carried out using the thermogravimetric process. This process measures the mass loss, mass and percentage of water in the sample being measured. This equipment is used in boost mode with the temperature to be reached set at 140°C.

III.2.4 Ash furnace - L15/11B410 Nabertherm

The L15/11B410 Naberthem ash furnace is used to calcine glassware. The standard conditions applied is 550°C± 25°Cs for 4h.

III.2.5 Reagents

Iron(II) sulfate heptahydrate ($FeSO_4.7H_2O$, CAS:7782-63-0); Sulfuric acid 72% (H_2SO_4, CAS: 7664-93-9); Hydrogen peroxide 30% (CAS: 7722-84-1) and Acétone ACS reagent, (> 99.5%, CAS: 67-64-1) were purchased from Sigma-Aldrich.

Alumina filters (Al_2O_3) were used to filter and recover microplastics. They were supplied by Cytiva. They have a mesh size of 0.2 µm and a diameter of 25 mm, allowing optimum recovery of microplastics

III.2.6 Thermogravimetric analysis (GTA)

TGA was carried out using a Mettler TGA2 (Schwerzebbach, Switzerland) equipped with an XP5U balance. Samples were heated from 25 to 900°C with a temperature ramp of 10°C/min at an air flow rate of 50 mL/min. The results were analysed using STARe software. The level of inorganic material was obtained by measuring the percentage of material remaining at 900°C.

III.2.7 Scanning Electron Microscopy (SEM)

The SEM analyses were carried out using a Thermo Scientific phenom ProX SEM, a so-called desktop SEM. It has the following characteristics:

- Source: CeB_6
- Sample size: max. diameter 32 mm
- Magnification between 80 and 350,000
- Resolution < 8 nm (BSD)

SEM was used with the backscattered electron detector (BSD), which enables the sample to be observed according to the chemical contrast. To allow optimal observation of the samples, the acceleration voltage was chosen at 10 kV.

III.2.8 Nikon SMZ18 stereomicroscope: episcopic image acquisition

Analyses were carried out using the SMZ18 Nikkon stereomicroscope and *NIS Element AR* software.

- Definition of resolution values:
  - Fast (focus): 3x8bit - 2880x2048 pixels
  - Quality (Capture): 3x8bit - 2880x2048 pixels
- White balance with white sheet:
  - Analogue gain of 2.2x
  - Exposure time (ms) (> 30ms) to obtain red, green and blue intensities equal to 200
- Magnification X2 to create a mosaic covering the entire filter using the "scan large image" tool. The overlap rate is set at 15%.
- Magnification X5 to capture 10 images to study their representativeness.

Subsequently to mosaic capture, the images are processed using two methods.
1- The first method involves using *ImageJ (v1.5.3)* software. Particles were thresholded, segmented and counted according to the assigned threshold. The software detects particles optimally when they are at least 25 pixels in size, with 1 pixel corresponding to 0.3 µm.

The image processing protocol included (1) Delimiting the analysis area using an elliptical section, (2) Setting the analysis scale 3.33 pixels/µm, (3) Defining the threshold for segmentation by adjusting the 'brightness' threshold so that particles of interest (based on a manually defined particle surfaces) are selected (Figure 2)

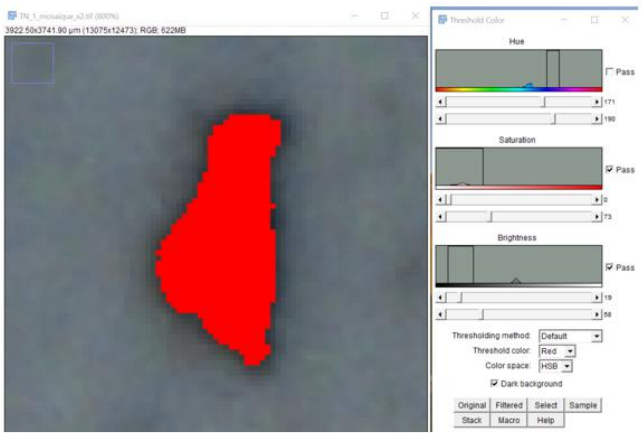


Figure 2: Threshold of colour to analyse the targeted microplastics

The analyse using *ImageJ* software allow particle characterisation with a minimum size of 25 pixels, which corresponds to approximately 7.5 µm². In the case of this study, the area range is defined as 10 µm² to infinite µm² (Figure 3).

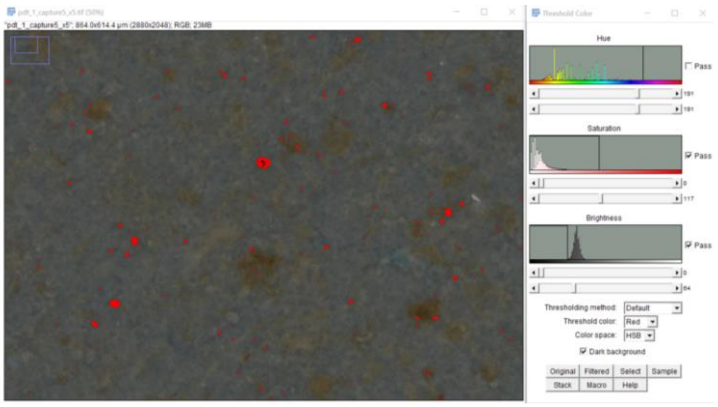


Figure 3: Visualization of targeted microplastics after the threshold of color

2- The mosaics were alternatively processed manually in order to quantify the impact of the automated particles selection of image treatment. Manual treatment was carried out using Paint 3D and consists of counting the microplastics considered as plastics (on a subjective criterion based on the colour of the particles and their morphology, two aspects that make it easy to distinguish plastic particles from organic residues by observing differences in rugged contour, density and transparency) by treating the images with various magnifications. A colour is assigned to each type of microplastic then transfer the segmented in mag on *ImageJ* to be used by performing a simple colour threshold. This thresholding enables the software to count the number of microplastics for each colour defined and requested. This procedure, carried out by eye, can be used to assess the presence of microplastics with a size < 10 µm², but is less accurate and may be subject to error.

III.3 Microplastics extraction method

III.3.1 Agro-residues pre-treatment

Potato peels were recovered using a steam peeling process. The samples (kept frozen) are thawed and dried for a week at 60°C to a water content of 5.2%. The peels were then ground using an SM300 knife grinder with a 2 mm grid.

The tomato pomace received from TOMA was dried in an oven at 60°C for 24 h, then ground successively in an SM300 knife mill (2 mm sieve) and an UPZ impact mill (0.5 mm sieve). The average particle size is D50~500 µm.

Grape pomace received from GrapeSud was dried in an oven at 60°C for 24 h, then ground successively in an SM300 knife mill (2 mm sieve) and an UPZ impact mill (0.5 mm sieve). The average particle size is D50~200 - 300 µm.

The brewer's spent grains came from a local brewery in Ieper, Belgium. Grain samples were dried at 105°C and then stored in plastic bags.

III.3.2 Prevention of contamination by microplastics in ambient air

Special precautions were taken during the experiments to avoid contamination of the samples by microplastics in the ambient air. All glassware used was first calcined in a muffle/ash furnace at 550°C for 4 hours. Spatulas and other laboratory utensils were systematically washed with acetone and then protected from any possible contamination.

III.3.3 Digestion protocol

Residues were preliminary submitted to an oxidative digestion using Fenton's reagent. 1g of sample was placed in an Erlenmeyer flask. A solution containing 10 mL of 30% hydrogen peroxide (H_2O_2) was added, followed by 1 mL of sulphuric acid (H_2SO_4) and finally 10 mL of iron (II) reagent ($FeSO_4.7H_2O$). The iron (II) reagent was produced by preparing a solution with a concentration of 0.05M $FeSO_4.7H_2O$. Triplicates were prepared for each type of agri-residues.

The Erlenmeyer flasks containing the digestion medium were placed in a bath with stirring, first in an ice bath and then the temperature was raised to 50°C. A negative control (using the reagents) was carried out in triplicate. The digestion scheme is illustrated in Figure 4.

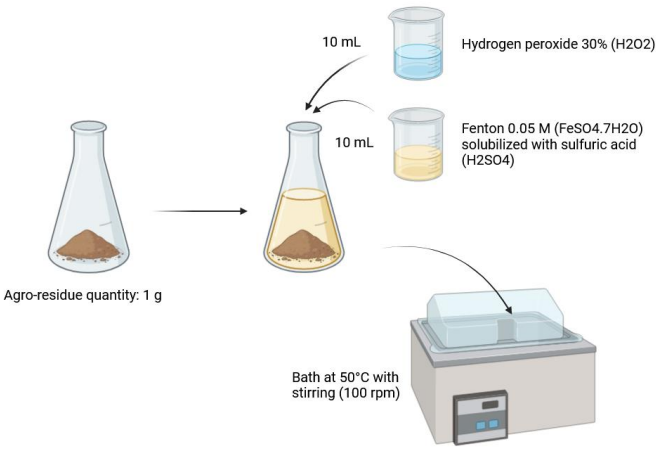


Figure 4: Digestion procedure applied to residue samples

The digestions were carried out over a period of seven days, with the addition of 10 mL of hydrogen peroxide and iron (II) solution every two days. A summary of the experiments is given in Table 10.

Table 10: Extraction protocol for various agro-residues

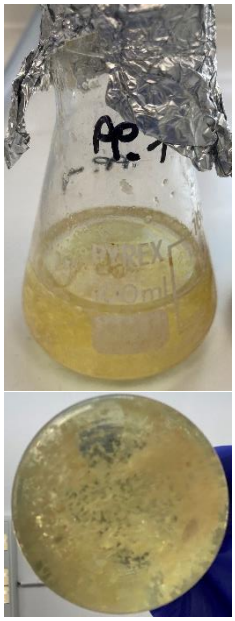
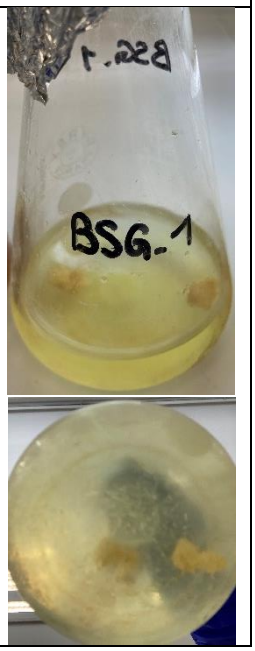
	Negative reference (without agri-residue)	Potato peels	Tomato pomace (seeds and peels)	Grape pomace (seeds, peels and branch)	Brewer's spent grain (BSG)
Digestion protocol	Day 1: 10 mL H_2O_2 + 10 mL $FeSO_4.7H_2O$ (0.05M) + 1 mL H_2SO_4 Day 3: 10 mL H_2O_2 + 10 mL $FeSO_4.7H_2O$ (0.05M) + 1 mL H_2SO_4 Day 5: 10 mL H_2O_2 + 10 mL $FeSO_4.7H_2O$ (0.05M) + 1 mL H_2SO_4				
Digestion condition	7 days – 50°C				

Pictures before digestion					

III.3.4 Filtration of digestates

At the end of the 7-day treatment period, the samples all showed a quantity of undigested material that had resisted treatment (Table 11).

Table 11: Digestion solutions after 7 days of digestion

	Negative reference (without any agro-residues)	Potato peels	Tomato pomace (seeds and peels)	Grape pomace (seeds, peels and branch)	Brewer's spent grain (BSG)
Pictures after digestion					

The previous separation between the digestate, the sediments and the un-digested parts of the agri-residue was performed using a 100 µm sieve (Figure 5). The retentates (residues and sediments) were recovered using a spatula then placed in petri dishes cleaned with acetone. Between each filtration, the sieve is rinsed with acetone to remove any particles (sediments and undigested agri-residues) remaining on the sieve filter. The residues obtained are shown in Figure 6.

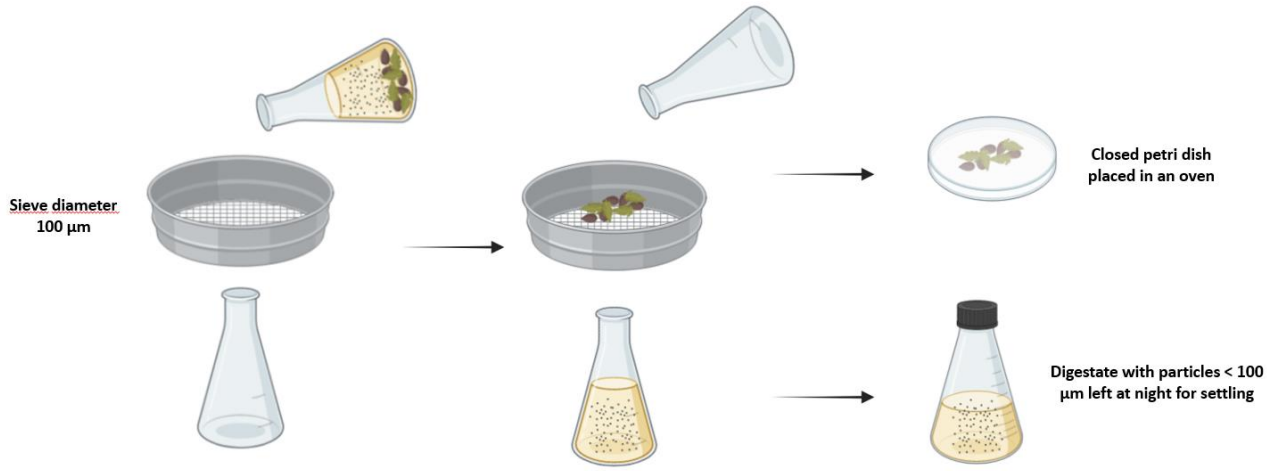


Figure 5: First filtration of the digestion solutions using a 100 µm sieve

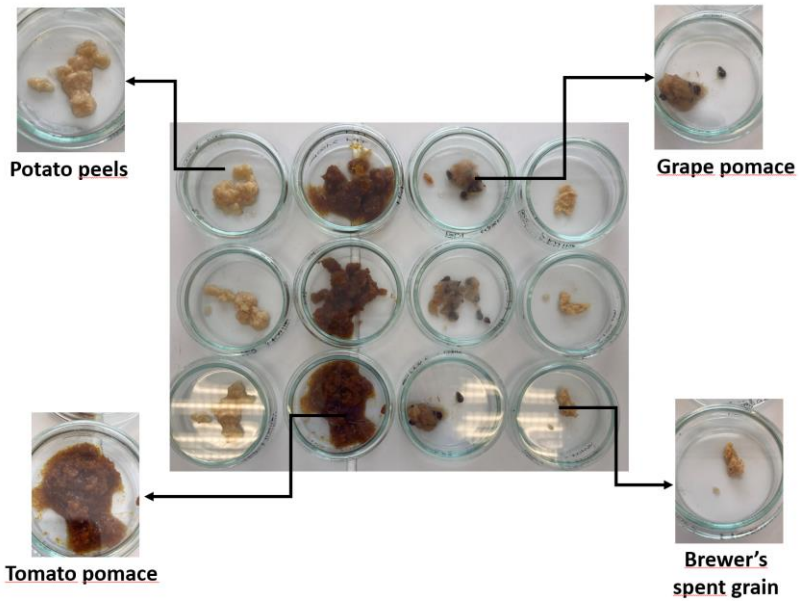


Figure 6: Non-digested part of the various agro-residues larger than 100 µm

The bottles containing sediment < 100 µm in size and digestate were left to decant for 24h in order to remove sediments in suspension which leads to clogging of the filter during the subsequent filtration stage (Figure 7). Supernatant filtration is carried out on an anodisc inorganic alumina filter (Al_2O_3) with a mesh size of 0.2 µm.

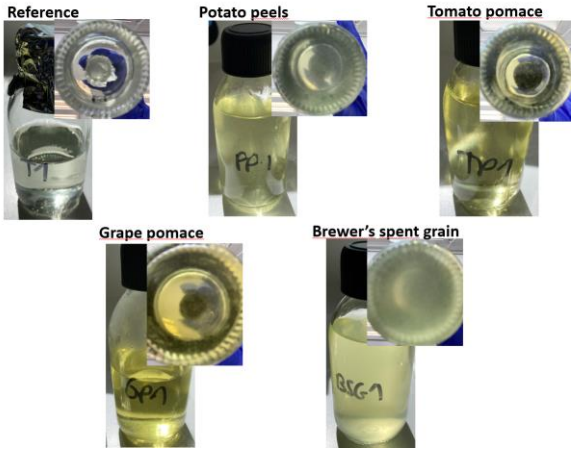


Figure 7: Digestion solutions after initial filtration during the decantation stage

After filtration, the filters are disposed of and preserved in petri dishes cleaned with acetone. To assess potential contamination by microplastics present in the ambient air, the petri dishes were divided into three sections to place the three filters (one filter correspond to one triplicate). This gives them their own space and limits their overlapping and potential contamination when they are transported to the analysis equipment.

Before the filtrations of the digestates, a blank control filter is placed in the center of the petri dish corresponding to each triplicate. This filter is used to assess the potential microplastic contamination due to the opening and closing of the petri dish when a filter is placed inside. The petri dishes before and after the second filtration are shown in Figure 8.

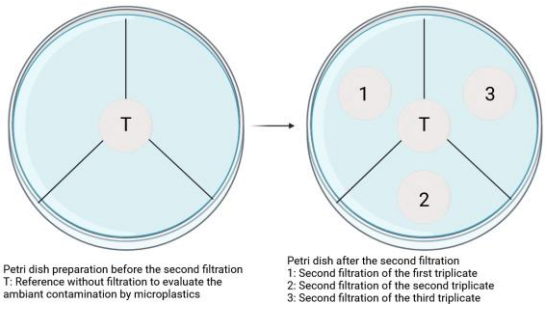


Figure 8: Petri dishes before (left) and after (right) second filtration

The bottle containing the digestate and the sediments that have decanted is carrefully tilted so that it filters the digestate without including the sediments. Once the digestate has been filtered, the alumina filter is recovered and placed in the dedicated petri dish. Once the filtration triplicates have been obtained for each agri-residue, the petri dishes are sealed with parafilm and left to dry at room temperature on a laboratory bench for at least 24 hours. The set-up for this second filtration is shown in Figure 9.

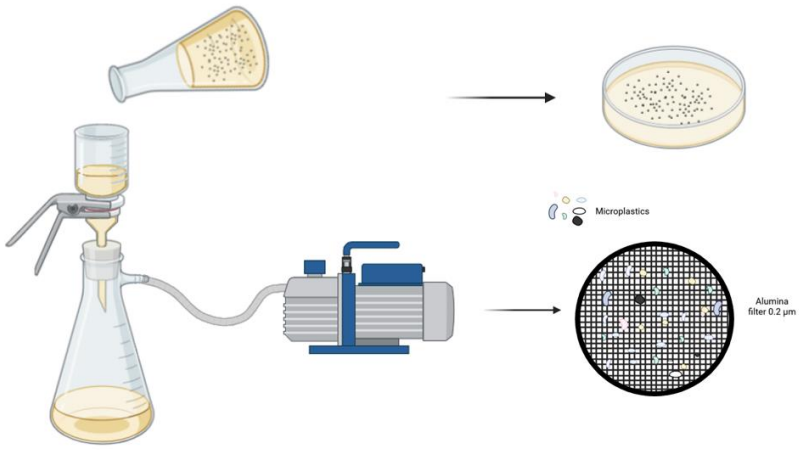


Figure 9: Second filtration and separation of digestate from sediments

Once the extraction protocol has been carried out, the products obtained are:

- Petri dishes with triplicate + blank control filter
- Petri dishes with undigested agri-residue + sediment larger than 100 µm
- Petri dishes with sediment < 100 µm in size

III.4 Results

III.4 .1 GTA measures on agro-residues

GTA analyses were carried out on the agro-residues before digestion in order to determine the proportion of inorganic matter. The analyses were carried out over a temperature range from 25 to 900°C with a temperature ramp of 10°C/min. These analyses were carried out under an air flow of 50 mL/min. The analysis results are presented in Table 12.

Table 12: GTA agro-residues measurements

	Inorganic mater rate *(% DM*)
Potato peels	8.4
Tomato pomace	0
Grape pomace	7.1
Brewer's spent grain	7.4

The agro-residues had similar levels of inorganic matter, except tomato pomace whose fractions are surprisingly devoid of mineral matter. The occurrence of inorganic compounds may be linked to the mineral content of the constituent tissues, but also to the presence of suspended sediment (or earth particles) that are resistant to digestion. This is a source of overestimation of the plastic particle count during image analysis. The presence of inorganic matter may explain the presence of suspended sediment in the digestion solutions which are a source of overestimation of the plastic particle count in image analysis.

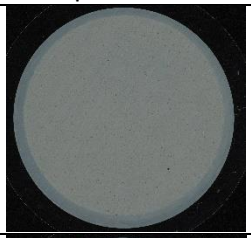
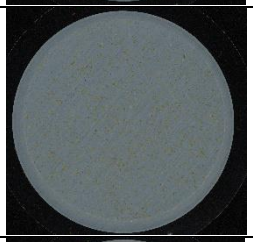
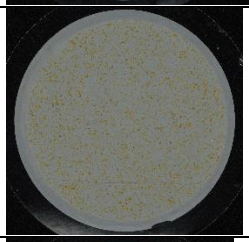
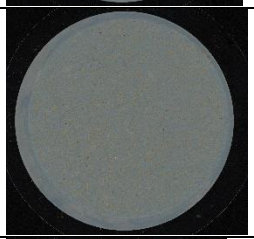
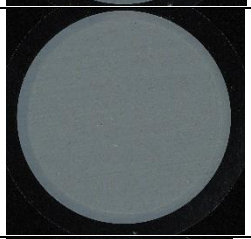
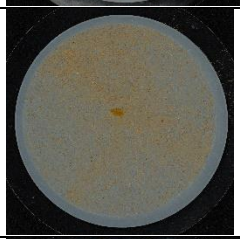
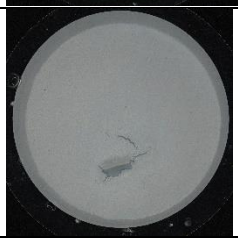
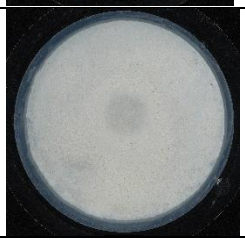
III.4 .2 Stereomicroscopic analysis and image treatment of agri-residue triplicates

The stereomicroscope images of the filters are shown in Table 13. It can be seen that the filters are covered almost entirely by a heterogeneous layer which seems to be composed of numerous particles, being a mixture of microplastics and undigested sediments smaller than 100 µm.

The presence of this layer indicates that during the second filtration stage, sediments are carried along with the microplastics into the filtration system. Their presence on the filter may indicate that some sediment remains in suspension in the digestate despite the prior decanting stage.

In the case of brewer's spent grain, the undigested residual matter forms a continuous film on the surface of the filter. The thickness of the layer is considerable, covering and masking numerous plastic particles. The second image in the triplicate is an illustration of this phenomenon, where the layer has broken, revealing the filter underneath with a few microplastics/particles on its surface. As consequence, the number of particles counted on these samples is undoubtedly underestimated.

Table 13: Stereomicroscopic images of filters from agricultural residue triplicates

	1st triplicate	2nd triplicate		3rd triplicate
Potato peels				
Tomato pomace				
Grape pomace				
Brewer's spent grain				

Taking this information into account, the microplastics were counted manually and using ImageJ for the different types of agri-residue and their values are reported in Table 14 (as indicated in the previous sections, white particles/microplastics were not counted)

Table 14: Quantity of microplastics particles/g of agri-residues

	ImageJ (p/m. > 10 µm²)	Manually (p/m. 0 - infiny µm²)
Potato peels	**499 ± 119.8**	**3327 ± 798.5**
Tomato pomace	365 ± 87.6	6254 ± 1501
Grape pomace	211 ± 50.6	978 ± 234.7
Brewer's spent grain	29 ± 7	36 ± 8.6

p/m.: particles/microplastics area (µm²)

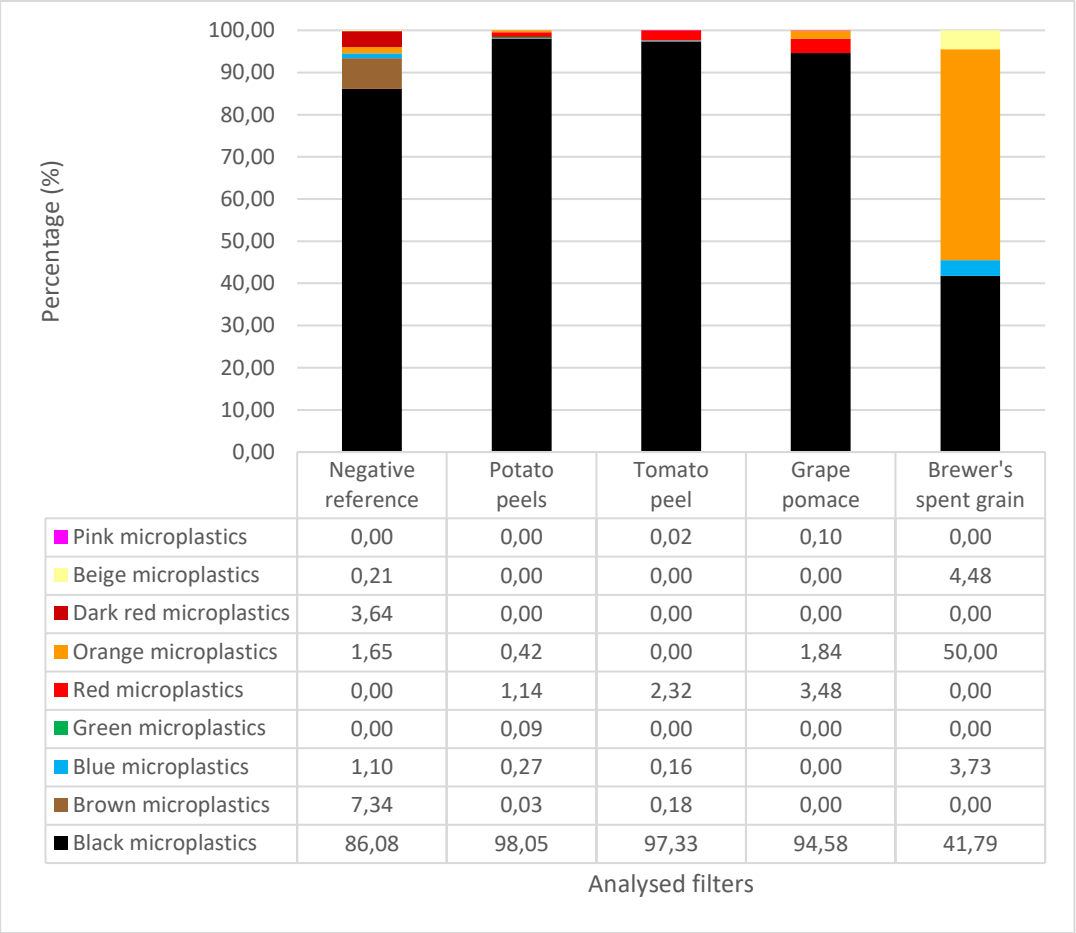
The number of microplastics counted is significantly more important using a manually selection because of the smaller scale analysed. The homogeneous and thick presence of sediments on the filter of the brewer's spent grain digestate seems to have an impact on the results. Depending on the fractions considered, these values are up to 100 times higher than those reported in the literature for whole fruit or tubers. As mentioned above, it is very difficult to compare values as they are highly dependent on the geographical and climatic conditions of the crop. It is also consistent to observe a higher level of contamination for residue fractions that are predominantly composed of the most peripheral tissues of the products and are therefore more exposed to airborne or soil contamination pathways (95-97).

The morphological properties of plastic particles are important characteristics that must be considered when studying their interactions with other organic and inorganic contaminants. The specific volume of the particles translated here by the circularity value reflects the specific surface area of the particle and then its exchange surface with the surrounding medium i.e its capacity to adsorb compounds (apart from any consideration of affinity). Resulting from automated image treatment, table 15 summarises the essential morphological properties of the fractions, highlighting both the major differences between the different residues and the great variability in shape/dimension within each fraction. These data simply and predictably reflect distinct contamination pathways for each fraction.

Table 15: morphological characteristics of particles from different residue fractions

	Area (µm²)	Ferret (µm)	Circularity
Potato peels	9,28 ± 40,75	3,63 ± 3,60	0,846 ± 0,15
Tomato pomace	23,98 ± 41,96	8,75 ± 4,48	0,333 ± 0,201
Grape pomace	19,54 ± 32,96	5,96 ± 4,18	0,715 ± 0,202
Brewer's spent grain	49,86 ± 85,13	12,32 ± 23,97	0,682 ± 0,233

The diversity of microplastics present in the agri-residues was analysed according to the colour of the particles (figure 10) on the basis of an analysis carried out manually.



	Negative reference	Potato peels	Tomato peel	Grape pomace	Brewer's spent grain
Pink microplastics	0,00	0,00	0,02	0,10	0,00
Beige microplastics	0,21	0,00	0,00	0,00	4,48
Dark red microplastics	3,64	0,00	0,00	0,00	0,00
Orange microplastics	1,65	0,42	0,00	1,84	50,00
Red microplastics	0,00	1,14	2,32	3,48	0,00
Green microplastics	0,00	0,09	0,00	0,00	0,00
Blue microplastics	1,10	0,27	0,16	0,00	3,73
Brown microplastics	7,34	0,03	0,18	0,00	0,00
Black microplastics	86,08	98,05	97,33	94,58	41,79

Figure 10: Percentage partition of microplastics colours for the different agri-residues

The agri-residues have many black microplastics of very small size (< 10 µm²) compared with the negative control which has few black microplastics but which are larger in size (> 10 µm²). This predominance of black particles suggests preferential contamination by agricultural mulch film, which is characteristically black in colour.

III.4.3 Size distribution of microplastics in the studied filter matrice

The figure 11 shows the coarse particle size distribution of particles from various fractions of residues based on Ferret diameter values. In all cases except for brewer's spent grain, the microplastics counted are smaller than 100 µm, which is consistent with the sieve used. The rare particles whose size exceeds this cut-off threshold are probably elongated fibres resulting from post-harvest contamination and whose morphology allows passage through the mesh of the sieve.

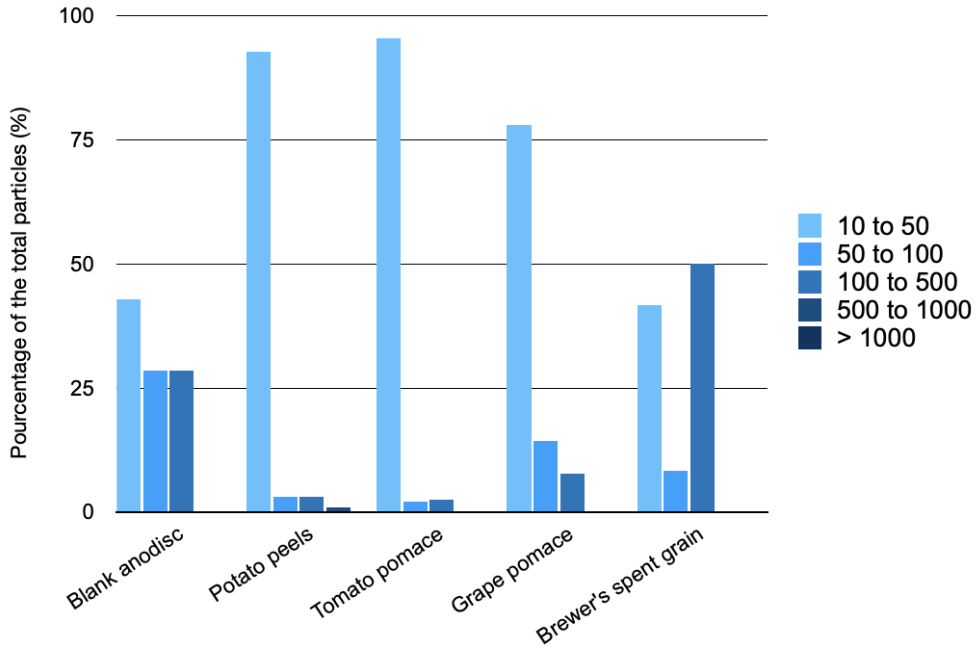


Figure 11: Size distribution of microplastics determined with ImageJ®

The highly predominant presence of particles smaller than 50 µm suggests contamination by particles smaller than 10 µm, which are not included in the count by the automatic ImageJ procedure. The different distribution observed in the specific case of brewer's spent grains is explained by the analysis difficulties identified and described previously, validating the hypothesis of significantly biased results due to the significant presence of organic matter in these samples.

III.5 Conclusion

Establishing a protocol for extracting and analyzing microplastics was complex since no standard method on the digestion of organic matter was up today available. The agri-residues considered in the Agriloop program have a complex composition (lignocellulosic fibres, inorganic matter, polysaccharides, cutin, suberin, etc.) which makes their digestion complicated. A number of oxidative and alkaline chemical digestion methods were tested following the protocols and conditions described in the literature, but proved ineffective as they did not achieve complete and totally satisfactory digestion of the samples. Developing a method for extracting and quantifying microplastics raises three major issues. The first concerned the prevention of potential contamination of samples by microplastics from various sources (air, reagents, glassware, etc.) throughout the microplastics extraction protocol. The second related to obtaining digestates (digested solutions) that could be filtered on 0.2 µm alumina filters and that could be analysed. Finally, the third challenge is to identify a viable and reliable analysis method for quantifying the number and the compositional nature of microplastics.

To address the first issue, contamination prevention was a success since the control filters, after filtration of the residues digestates, contained very few or no textile fibres (the main contaminant in the ambient air). To prevent contamination and check the analysis surface before filtration, blank alumina filters just removed from their container were analysed using a stereomicroscope and SEM.

These measurements showed that the filters had surface flaws which, on the scale of the image processing carried out, represented particles that could easily be confused with microplastics. This experimental bias therefore prevents white particles from being counted, resulting in a significant underestimation of plastic particle quantification.

The poor digestibility of the residues fractions has led to the implementation of a large number of experiments aimed at reducing the quantity of residual matter. In spite of numerous trials carried out to improve digestion, separate the sediments from the digestate and improve filtration capacity, the digestion remains incomplete and result in main subsequent analytical limits. The first limitation is obviously the robustness of the particle count, as the presence of organic debris may lead to an overestimation of plastic particle contamination, the extent of which has yet to be assessed. The second limitation concerns the application of compositional particle analysis techniques. The residual organic matter is probably composed of lignocellulosic material in the case of potato peels and brewers' grains, and of waxy substances in the case of tomato pomace. These compounds are present in the form of a superficial layer that coats the plastic particles, preventing them from being analysed by FT-IR microspectroscopy. FTIR transmission measurements resulted in spectra that were difficult to interpret due to the combination of polymer signatures and organic compounds, preventing identification of the nature of the materials.

The results presented at this level remain preliminary and the method developed needs to be consolidated and subjected to further meteorological analysis. Nevertheless, and despite these operational limitations, image analysis carried out on the four types of sample showed systematic and obvious contamination by plastic particles. The results also show that the image processing methods used produce results that are consistent with the analysis capacities of the two methods, which responds to the third issue. The combined approach using both automated and manual particle characterisation showed that working on two scales is complementary because it provides access to information on microplastics in terms of size distribution, colour distribution and quantity according to the different sizes of microplastics.

The contamination extent clearly depends on the observation scale used with a factor of 10 between measurements made with ImageJ with a particle size > 10 µm² and measurements made manually, considering all particles having a minimum microplastic size of 2-3 pixels, which corresponds to approximately one micrometer. The coefficient of variation of 24% (calculated on the triplicates) agrees with the common value observed in literature data.

With a view to elucidating the mechanisms by which microplastics interact with other contaminants (pesticides and mycotoxins), more detailed characterisation of these plastic particles is still required. To this end, and with a view to further work under the Agriloop programme, additional analyses will be carried out using pyrolysis coupled with GC-MS . GC-pyrolisis analysis provides an overall measure of the extent of plastic contamination without providing a coupled (morphology/composition) particle-by-particle analysis. This technique appears to be well suited to the needs of the study, since it avoids the analytical problems associated with the presence of organic matter.

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Data Management Plan follow-up

N°	Dataset name	Open Data	Closed Data	Means of dissemination	Maximum delay before access	Data set access
	WP1- Task 1.3 UNIROMA (Partner #12)	Data have been elaborated in excel files, available on request in the AgriLoop Dataverse.	Data reported in the deliverable are available on request	Scientific publications, International and national conferences and dissemination events	Once published and at the latest 2 years after the end of the project	https://doi.org/10.57745/2UTXQY (dataset still to be reviewed)

This table above sums up the main information regarding the data produced for this deliverable, where is it stored and are the specific rules to respect concerning access, publication and FAIR principles.

N°	Dataset name	Owner	Name of the current contact	IPR issues	Use of third-party	Restrictions on data sharing (Y/N)
	WP1- Task 1.3 UNIROMA (Partner #12)	UNIROMA	Angela Marchetti Chiara Cavaliere Marianna Villano	To be defined according to the Institution regulation	no	Yes, compliance with GDPR

This table above sums up the main information regarding potential Intellectual property protection or GDPR issues.

Annex I - Analysis of mycotoxins

Zearalenone, Fumonisin and Enniatin families

	Tomato grains	Tomato peels	tomato pomace	BSG	Potato peels
Mycotoxins	µg/kg dried material	µg/kg dried material	µg/kg dried material	µg/kg dried material	µg/kg raw material
Zéaralénone	<30	<50	<30	14	<10
Alpha Zéaralanol	<75	<125	<75	<25	<25
Alpha Zéaralénol	<30	<50	<30	<10	<10
Béta Zéaralanol	<75	<125	<75	<25	<25
Béta Zéaralénol	<30	<50	<30	<10	<10
Fumonisine B1	<30	<50	<30	<10	<10
Fumonisine B2	<30	<50	<30	<10	<10
Fumonisine B3	<30	<50	<30	<10	<10
Beauvericin	<30	<50	<30	<10	<10
Enniatin A	<15	<25	<15	8	<5
Enniatin A1	<15	<25	<15	55	<5
Enniatin B	<15	25	9	320	<5
Enniatin B1	<15	<25	<15	180	<5

Trichothecene family

	Tomato grains	Tomato peels	tomato pomace	BSG	Potato peels
Mycotoxins	µg/kg dried material	µg/kg dried material	µg/kg dried material	µg/kg dried material	µg/kg raw material
DON	<30	<50	<30	<10	<10
15 ac DON	<30	<50	<30	<10	<10
3 ac DON	<30	<50	<30	<10	<10
DON 3G	<60	<100	<60	<20	<20
DAS	<30	<50	<30	<10	<10
DOM-1	<60	<100	<60	<20	<20
Nivalénol	<30	<50	<30	<10	<10
Fusarénone X	<30	<50	<30	<10	<10
T-2	<30	<50	<30	<10	<10
HT-2	<30	<50	<30	<10	<10
T-2 Tetraol	<150	<250	<150	<50	<50
T-2 Triol	<150	<250	<150	<50	<50
MAS	<30	<50	<30	<10	<10

Alternaria family and Ergot alkaloids

	Tomato grains	Tomato peels	tomato pomace	BSG	Potato peels
Mycotoxins	µg/kg dried material	µg/kg dried material	µg/kg dried material	µg/kg dried material	µg/kg raw material
Acide ténuazonique	<60	<100	<60	<20	<20
Altenuène	<30	<50	<30	<10	<10
Alternariol	<150	<250	<150	<50	<50
Altertoxin	<150	<250	<150	<50	<50
AME	<15	35	<15	<5	<5
Tentoxin	<15	<25	<15	<5	<5
Ergocornine	<15	<25	<15	<5	<5
Ergocorninine	<15	<25	<15	<5	<5
Ergocristine	<15	<25	<15	<5	<5
Ergocristinine	<15	<25	<15	<5	<5
Ergocryptine	<15	<25	<15	<5	<5
Ergocryptinine	<15	<25	<15	<5	<5
Ergometrine	<15	<25	<15	<5	<5
Ergometrinine	<15	<25	<15	<5	<5
Ergosine	<15	<25	<15	<5	<5
Ergosinine	<15	<25	<15	<5	<5
Ergotamine	<15	<25	<15	<5	<5
Ergotaminine	<15	<25	<15	<5	<5

Ochratoxins and aflatoxins family and others mycotoxins produced by Aspergillus and Penicillium

	Tomato grains	Tomato peels	tomato pomace	BSG	Potato peels
Mycotoxins	µg/kg dried material	µg/kg dried material	µg/kg dried material	µg/kg dried material	µg/kg raw material
Ochratoxine A	<3	<5	<3	<1	<1
Ochratoxine alpha	<15	<25	<15	<5	<5
Ochratoxine B	<15	<25	<15	<5	<5
Aflatoxine B1	<3	<5	<3	<1	<1
Aflatoxine B2	<3	<5	<3	<1	<1
Aflatoxine G1	<3	<5	<3	<1	<1
Aflatoxine G2	<3	<5	<3	<1	<1
Verruculogen	<150	<25	<150	<50	<50
Acide cyclopiazonique	<150	<250	<150	<50	<50
Citrinine	<300	<500	<300	<100	<100
Patuline	<30	<50	<30	<10	<10
Roquefortin C	<15	<25	<15	<5	<5
Sterigmatocystin	<15	<25	<15	<5	<5