D4.3 Report on the development of matrix-specific sample preparations

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1 Executive Summary

A specific preparation procedure was developed to allow filtration over the *Sieve*-ID® microsieve membranes for each sample matrix. Matrix-specific interferences were identified, and matrix clean-up procedures (removal of debris and filtration/detection-inhibiting components from the sample) were developed. The recovery rates of the microbial and chemical analytes were determined for each sample preparation method. A single sample preparation strategy for both biological and chemical analytes was developed for the aquaponics waters and honey matrices. Sample preparation for aquaponics water consisted of a simple pre-filtration using a 10-micron syringe filter to remove sludge and algae. Honey samples were first diluted using 1× PBS (pH7.4) before pre-filtration using a 5-micron syringe filter to remove excess yeast cells and fungal spores. The developed sample preparation strategies improved filtration volumes over Sieve-ID® microsieve membranes, while both biological and chemical analytes showed high recovery values. However, different sample preparation strategies were required for analyte detection in raw milk and beer; one for the biological analytes and one for the non-biological analytes.

Especially for the biological targets, filtration and consequent concentration of low numbers of microbes over the microsieve membranes is essential. Removal of filtration-blocking components with no loss in microbial cell counts was incompatible with recovery of the chemical analytes due to the inclusion of a centrifugation step. Removal of the supernatant leads to the loss of the chemical analytes. For the chemical analytes, the only requirement to overcome matrix interference for raw milk and beer is sample dilution in a buffer. Samples can be directly loaded into the h-ALO sample cartridge and would not require filtration over the *Sieve*-ID® microsieve membranes.

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2 Development of sample preparation protocols

2.1 Identification of filtration-inhibiting compounds

The most likely matrix-specific interferences for filtration over microsieves were identified to develop effective sample preparation procedures. Aquaponic waters may contain sludge, yeasts, fungi, algae and other organic materials, which limit the filtration over the microsieve membranes. Additionally, aquaponics water may contain high numbers of microbial background flora next to the microbe of interest, potentially clogging the membrane filters. Honey is a supersaturated substance composed mainly of the sugars glucose and fructose. These substances tend to crystallize when dissolved further, which, during filtration, can block the membranes. Size-based filtration hampering ingredients of honey include pollen, dust, yeast and fungal spores. Raw milk contains fat, proteins and lactose as filtration-inhibiting compounds. The quantities of these raw milk constituents can vary considerably depending on environmental conditions, the individual animal, its breed, stage of lactation, age and health status. Craft beer contains many living yeast cells to facilitate bottle conditioning, limiting the filtration. Depending heavily on the beerstyle and types of malts and yeast used, beers contain potential microsieve-blocking constituents like carbohydrates, starch, proteins and remnants of creative brewing ingredients like, e.g. coffee, cacao or fruits.

Table 1. Filtration inhibiting compounds per matrix.

Matrix	Potentially filtration inhibiting compounds	Selected microsieve pore size
Aquaponic water	Algae, bacterial background flora, sludge, plant material, yeast, fungi	0.45 µm
Honey	Fungal spores, yeasts, pollen, sugars (glucose and fructose)	3.0 µm
Raw milk	Fat, proteins/mycells, lactose, bacteria	3.0 µm
Beer	Carbon dioxide, carbohydrates, starch, proteins, yeasts, remnants of creative brewing ingredients	0.45 µm

2.2 Sample preparation and recovery of microbiological analytes from aquaponic water

Two bottles of aquaponic water were provided by The Circle. Samples were first filtered without sample preparation using 0.45µm *Sieve*-ID microsieve membranes. An average of 9 mL of Aquaponic water could be filtered using the microsieves with filtration times of 10 min or longer (Table 2). Large filtration-hampering structures like algae were visible in the aquaponic water samples without pre-treatment (Figure 1A-C). After pre-filtration using a 10µm Acrodisc PSF Versapor syringe filter, at least 10 mL of both aquaponic waters could be filtered using 0.45µm *Sieve*-ID microsieve membranes within 5 minutes. In the aquaponics water samples with pre-treatment, large filtration-hampering structures like algae were absent (Figure 1D), facilitating the speed and volume of filtration.

Next, the microbial analyte recovery rate of the sample pre-treatment was tested. First, 1× PBS (pH 7.4) and an autoclaved 50:50 mixture of aquaponics water bottles 1 and 2 were spiked with a 10-fold dilution series of *E. coli* bacteria. Samples were analyzed for microbial counts with and without the 10µm syringe filterdisc filtration sample pre-treatment. Figure 2 shows that the microbial recovery rates after sample pre-treatments were equally high for the 1× PBS (pH 7.4) and the aquaponic water samples for all the tested *E. coli* concentrations. Recovery rates of >87% were achieved in all cases indicating that matrix components from aquaponics waters did not influence microbial recovery rates. Finally, the recovery rates for naturally occurring microbes in the two aquaponics water samples were tested. As shown in Figure 2, the microbial recovery rates of >91% for both aquaponics water samples showed minimal losses of microbial analytes due to the sample preparation steps, further indicating the robustness of the sample pre-treatment.

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Table 2. Filtration of aquaponics waters over 0.45µm *Sieve*-ID microsieve membranes.

Sample	Without sample pre-treatment		With sample pre-treatment	
	Volume (SD)	Ave time (min)	Volume (SD)	Time (min)
Undiluted Aquaponic water bottle 1	8,7 mL (0,3)	14	11,25 mL (0,25)	<5 Min
Undiluted Aquaponic water bottle 2	8,8 mL (0,4)	16	11,33 mL (0,38)	<5 Min

Experiments were performed in triplicate. SD: standard deviation

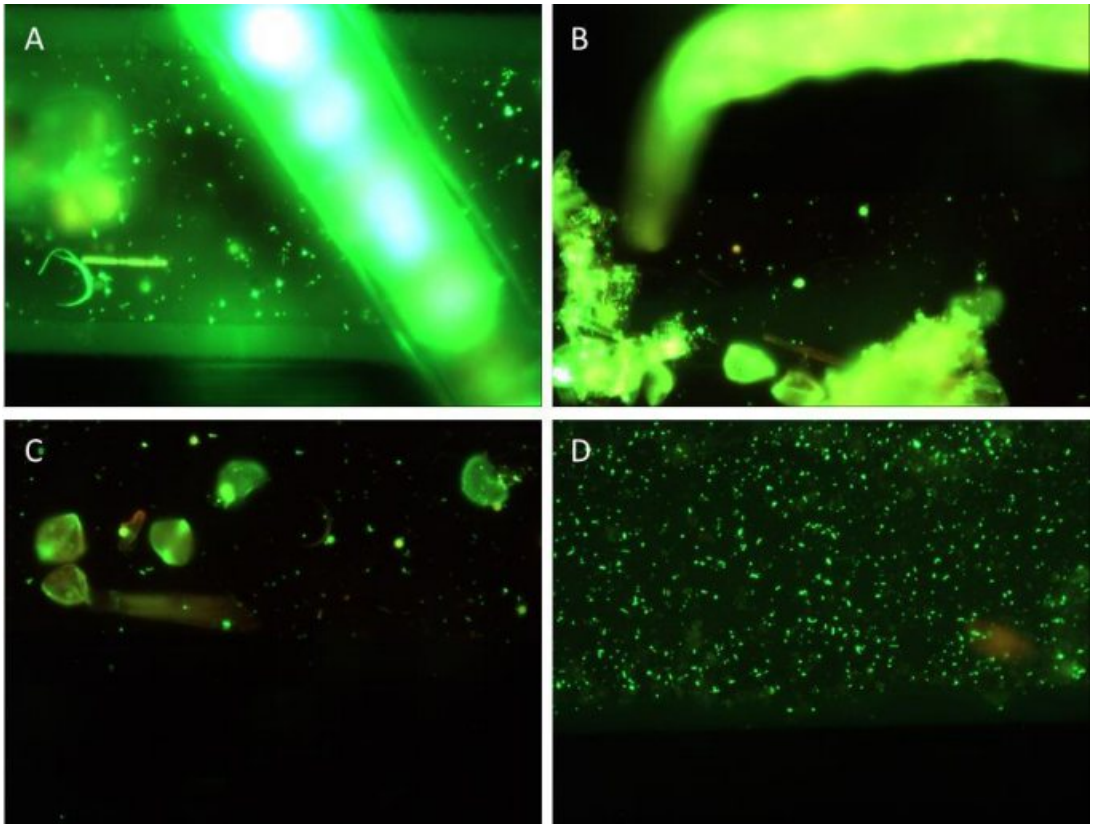

Figure 1. Effect of sample pre-treatment of Aquaponic waters. **A**, **B** and **C:** no sample pre-treatment. 2 mL Aquaponics water was directly filtered using *Sieve*-ID microsieve membranes. Large green fluorescent spots show background structures like algae, yeasts, fungi and plant materials. Small green fluorescent spots indicate bacteria. **D:** sample pre-treatment by pre-filtration using a 10µm Acrodisc PSF Versapor syringe filter. 10 mL Aquaponics water filtered using *Sieve*-ID microsieve membranes. Large green fluorescent spots are absent, while the small green fluorescent spots indicate bacteria. All samples are stained with the *Sieve*-ID total viable count kit.

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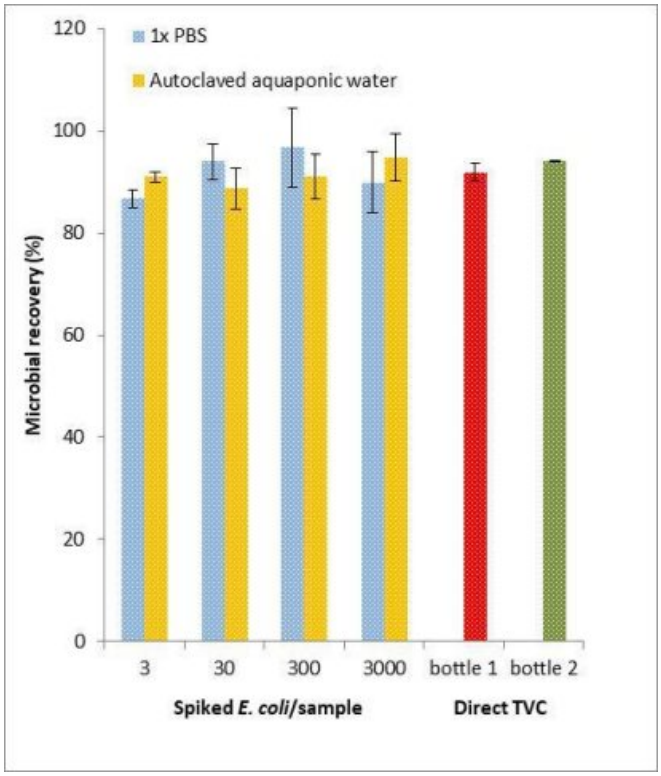

Figure 2. Microbiological recovery rates after sample pre-treatment of Aquaponic waters. 1× PBS (pH 7.4) (**Blue**) and an autoclaved 50:50 mixture of aquaponics water bottles 1 and 2 (**Orange**) spiked with a 10-fold dilution series of E. coli bacteria (3, 30, 300 and 3000 CFU/tested sample). The recovery rates for naturally occurring microbes in Bottle 1 (**Red**) and bottle 2 (**Green**) were tested. Samples were analyzed for microbial counts with and without the 10µm syringe filterdisc filtration sample pre-treatment using the *Sieve*-ID total viable count kit. All samples were tested in triplicate.

2.3 Sample preparation and recovery of microbiological analytes from honey

A biological Acacia honey sample was purchased in Het Bijenhuis (the Beehouse) in Wageningen. The honey sample had to be diluted at least 10× (w/v) in pre-warmed (37˚C) 1× PBS (pH 7.4) before any easy sample handling, and filtration was possible. Sample pre-treatment consisted of a 5 µm or a 10µm Acrodisc PSF Versapor syringe filtration step. Further sample pre-treatment using amylase enzymes did not further improve filtration (data not shown). When the honey was filtered using 0.45 µm *Sieve*-ID microsieve membranes, the membranes blocked almost instantly, and no filtration was possible. When filtered using 3.0 µm *Sieve*-ID microsieve membranes, large-volume handling was possible depending on the applied sample pre-treatment (Table 3). Without pre-filtration, filtration hampering structures like spores and/or pollen are clearly visible (Figure 3), while pre-filtration removed most of these structures. Additionally, the sample dilution factor did not influence the filterable amount of honey over the *Sieve*-ID microsieve membranes.

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Table 3. Filtration of honey over *Sieve*-ID microsieve membranes.

Sample	*Sieve*-ID microsieve membranes pore size	No sample pre-treatment	Sample pre-treatment	
			5.0µm	10.0µm
		Filtered honey (gram)	Filtered honey (gram)	Filtered honey (gram)
honey diluted 10×	0.45µm	<10µg	<10µg	<10µg
honey diluted 100×	0.45µm	<10µg	<10µg	<10µg
honey diluted 10×	3.0µm	0.2	12.5	1.6
honey diluted 100×	3.0µm	0.2	12.75	1.8

All honey samples were 10× or 100× (w/v) diluted in pre-warmed (37 ˚C) 1× PBS (pH 7.4). Filtrations of honey samples over the 3.0µm Sieve-ID microsieve membranes were performed in triplicates.

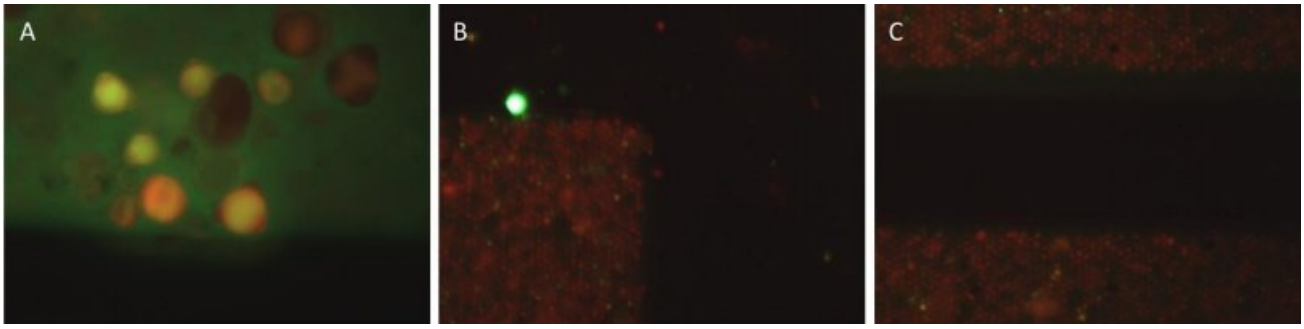

Figure 3. Effect of sample pre-treatment of honey samples. **A**: no sample pre-treatment. 2 mL 10× diluted honey (0.2 grams) was directly filtered using a 3.0µm *Sieve*-ID microsieve membrane. Background structures like spores and/or pollen are visible. **B:** sample pre-treatment using a pre-filtration using a 10µm Acrodisc PSF Versapor syringe filter. 15 mL 10× diluted honey (1.5 grams) was filtered using a 3.0µm *Sieve*-ID microsieve membrane. Spores and pollen are absent, and the large green fluorescent spot indicates a viable yeast cell. **C:** sample pre-treatment using a pre-filtration using a 5µm Acrodisc PSF Versapor syringe filter. 120 mL 10× diluted honey (12 grams) was filtered using a 3.0µm *Sieve*-ID microsieve membrane. No spores, pollen or yeast cells were visible. All samples are stained with the *Sieve*-ID total viable count kit.

For the microbial recovery rate experiments, honey samples were diluted 10× and 100× (w/v) in pre-warmed 1× PBS (pH 7.4), autoclaved and spiked with a 10-fold dilution series of *B. cereus* bacteria before sample pre-treatment using syringe filters. Figure 4 shows that microbial recovery rates did not differ between the spiked bacterial numbers. They were independent of the honey dilution factor or the presence of the honey matrix. The bacterial recovery rates were 61.6 % (StDev 2.0%) after the 5µm syringe filtration step, while after the 10µm syringe filtration step, bacterial recovery rates were 91.7% (StDev 1.9%). However, this loss in microbial recovery rate was easily compensated by the 10-fold higher filtration volumes of the 5 µm micron pre-filtered samples over the microsieve membranes.

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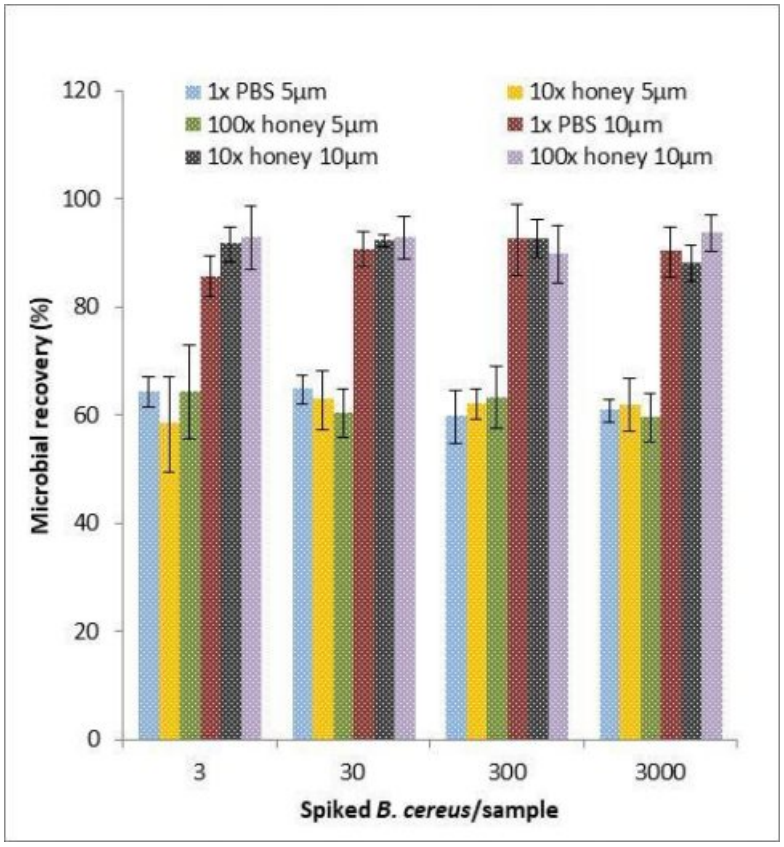

Figure 4. Microbiological recovery rates after sample pre-treatment of honey. 1× PBS (pH 7.4) (**Blue**), 10× (w/v) diluted honey (**Yellow**), 100× (w/v) diluted honey (**Green**) were spiked with a 10-fold dilution series of *B. cereus* bacteria (3, 30, 300 and 3000 CFU/tested sample) and pre-filtered over 5µm syringe filterdiscs. 1× PBS (pH 7.4) (**Red**), 10× (w/v) diluted honey (**Black**), 100× (w/v) diluted honey (**Purple**) were spiked with a 10-fold dilution series of *B. cereus* bacteria (3, 30, 300 and 3000 CFU/tested sample) and pre-filtered over 10µm syringe filterdiscs. All samples were also analyzed for microbial counts without the syringe filterdisc sample pre-treatments. All samples were tested in triplicate.

2.4 Sample preparation and recovery of microbiological analytes from raw milk

Raw milk samples were purchased at three local farms around Wageningen. Each raw milk sample was sampled from a temperature-controlled milk tank and was a maximum of three days old at the moment of sampling. Raw milk without sample pre-treatment was completely unfilterable over 0.45 µm and 3.0µm *Sieve*-ID microsieve membranes (Table 4). Tested sample pre-treatment strategies consisted of chemical treatments (e.g. chelating agents and surfactants), syringe filtration using 5µm Acrodisc PSF Versapor syringe filters and enzymatic treatments (Proteinase-K). Lipases were also tested as enzymatic treatments, but the application of lipases killed the microbial analytes in the sample (data not shown). Also, combinations of the treatments were tested. In none of the treatments, the samples could be filtered in sufficient volumes over 0.45µm *Sieve*-ID microsieve membranes (data not shown). For filtration using 3.0µm *Sieve*-ID microsieve membranes, larger volumes of raw milk samples could be filtered after a combination of centrifugation, pre-filtration and enzymatic treatment. Differences in filterable volumes were observed among the raw milk farms and samples from the same farm taken on different dates (Table 4), indicating that the consistency of raw milk samples was highly variable.

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Table 4. Effect of sample pre-treatment on filtration volumes of raw milk over the *Sieve*-ID microsieve membranes.

Raw-Milk sample	Sampling date	Direct	Syringe pre-filtration (5µm)	centrifugation (2800×*RCF*)	Chemical + Enzymatic treatment	Centrifugation + Chemical/Enzymatic treatment + Syringe pre-filtration
Milkfarm 1	20-4-21	<50µl	<100µl	<100µl	100µl	30 mL
	17-08-21	<50µl	<50µl	<100µl	<100µl	14 mL
	24-5-22	<50µl	<50µl	<100µl	<100µl	10 mL
Milkfarm 2	20-4-21	<50µl	50µl	<100µl	<100µl	24,5 mL
	17-08-21	<50µl	50µl	<100µl	100µl	18 mL
Milkfarm 3	21-4-21	<50µl	<50µl	<100µl	<100µl	4 mL

Samples were analyzed in duplicate. Milk samples were centrifuged at 2800×RCF for 15 minutes. Enzymatic treatments were performed with Proteinase-K and Amylase for 30 min at 37˚C in a background of 0.25×PBS pH7.4. The indicated milk filtration volumes were corrected for dilution. All samples were filtered over 3.0 µm Sieve-ID microsieve membranes.

Next, the analyte recovery rate of the sample pre-treatment consisting of centrifugation + chemical/enzymatic treatment + syringe pre-filtration was tested. To visualize the recovery rates without raw milk matrix components, 1× PBS(pH7.4) samples were spiked with a 10-fold dilution series of *Salmonella enterica subsp. Enterica serovar Thompson* bacteria before sample pre-treatment. As shown in Figure 6B, the microbial recovery rates varied between 15% (StDev 5.5%) and 20% (StDev 4.3%) in the absence of the milk matrix. Because raw-milk samples often naturally contain more than 10.000 CFU/mL, samples were not additionally spiked with *Salmonella* bacteria, and the total microbial recovery was determined. Figure 5 shows that a thick, hard layer of buttery fat formed on top of the milk surface/supernatant after sample centrifugation. This layer proved challenging to remove without leaving residual fat in the sample during the removal of the supernatant. The pellet was dissolved in a 10mL 1× PBS pH7.4 buffer containing a low concentration of a surfactant, chelating agent and Proteinase-K. The mixture was incubated for 30 minutes at 37 ˚C before pre-filtration using a 5µm Acrodisc PSF Versapor syringe filter. Samples before and after sample preparation were analyzed on TSA plates with cycloheximide to minimize the Eukaryotic cell growth. As shown in Figure 6A, the observed recovery rates were between 8.5% (StDev 1.5%) and 31.3% (StDev 3.7%), indicating that the milk matrix influenced microbial recovery. Even though the microbial cell recoveries were relatively low, the increased filtration volumes of milk are more than sufficient to compensate for the loss of microbial recovery.

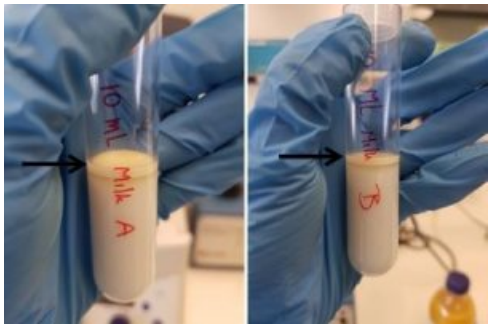

Figure 5. Examples of the thick, hard layer of buttery fat formed on top of the milk surface/supernatant after sample centrifugation. The thick layer is indicated by the black arrows.

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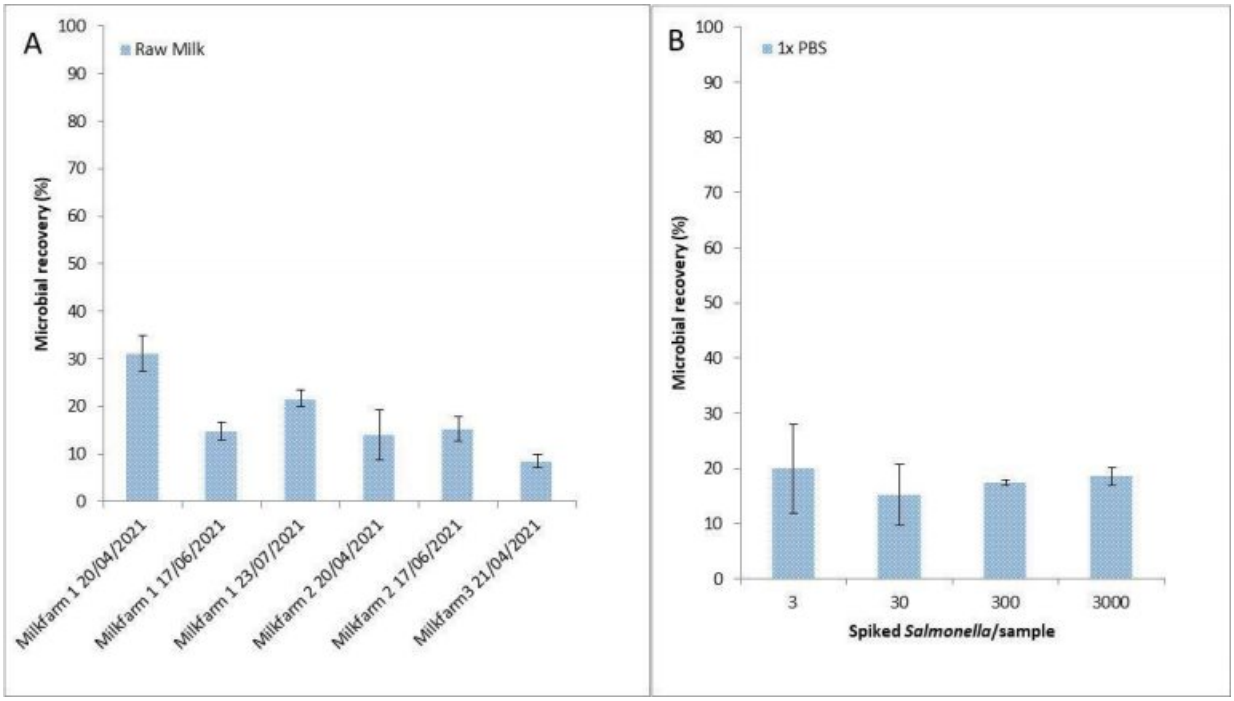

Figure 6. Microbiological recovery rates after sample pre-treatment of raw-milk samples. **(A)** The recovery rates for naturally occurring microbes in different milk samples. Samples were analyzed for microbial counts with and without sample pre-treatment using TSA plates with cycloheximide to minimize the Eukaryotic cell growth. All samples were tested in triplicate. **(B)** 1× PBS (pH 7.4) spiked with a 10-fold dilution series of *Salmonella enterica subsp enterica serovar Thompson* bacteria (3, 30, 300 and 3000 CFU/tested sample).

2.5 Sample preparation and recovery of microbiological analytes from beer

A range of different beer samples was purchased in the local shops in Wageningen or obtained directly from breweries. The beers could be classified into 7 distinct groups/beer styles: Pilsner, Blond, India Pale Ale (IPA), Stout, Barleywine and New England IPA (NEIPA). Because many craft beers are unfiltered and contain many yeast cells, sample pre-treatment always consisted of a 5 µm Acrodisc PSF Versapor syringe filtration step to reduce the number of yeast cells. A very high variation of filtration volumes was observed between different beer styles when filtered using 0.45 µm *Sieve*-ID microsieve membranes (Table 5). Wheras Pilsners, Blond and IPA had good filtration volumes, for Stouts, Barleywines and NEIPAs low filtration volumes were achieved. All three low-volume filtering styles have high sugar and/or protein content. An enzymatic step containing optimized concentrations (data not shown) of amylase and proteinase-K was introduced in a background of 0.25×PBS pH7.4. After enzymatic treatment, all beer styles showed improved filtration volumes; however, the filtration volumes of the Stouts and NEIPAs remained relatively low. Introducing an additional centrifugation step sometimes, but not consistently, yield better filtration results (Table 5).

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Table 5. Effect of sample pre-treatment on filtration volumes of beer over the *Sieve*-ID microsieve membranes.

Beer	Direct	Syringe pre-filtration (5µm)	Syringe pre-filtration (5µm) + Enzymatic treatment	Additional centrifugation (10000×*RCF*)
Pilsner	15mL	15ml	18mL	18mL
IPA 1	3.5mL	7.0mL	12mL	12mL
IPA 2	4.0mL	8.5mL	13mL	N.T.
Blond	400µl	8.0mL	12mL	N.T.
NEIPA 1	100µl	200µl	1mL	1.8mL
NEIPA 2	100µl	150µl	450µl	900µl
NEIPA 3	100µl	125µl	800µl	750µl
Stout 1	150µl	150µl	500µl	1.5mL
Stout 2	100µl	100µl	250µl	N.T.
Barleywine/Quad 1	125µl	650µl	6mL	5.8mL
Barleywine/Quad 2	200µl	400µl	9mL	N.T

Samples were analyzed in triplicate. Standard deviations were minimal and are, therefore, not indicated. Enzymatic treatments were performed with Proteinase-K and Amylase for 30 min at 37˚C in a background of 0.25×PBS pH7.4. The indicated beer filtration volumes were corrected for dilution. All samples were filtered over 0.45 µm Sieve-ID microsieve membranes. N.T. not tested.

The Pilsner, IPA and Barleywine samples were selected as matrices for the microbial recovery experiments and were spiked with 10-fold dilution series of *Lactobacillus brevis* before sample pre-treatment. To visualize the recovery rates without beer matrix components, 1× PBS (pH7.4) samples were also spiked with a 10-fold dilution series of *Lactobacillus brevis* before sample pre-treatment. As shown in Figure 7, all samples showed microbial recovery rates between 66.6% (StDev 6,5) and 78.8% (StDev 5.3%). The microbial recovery rates did not differ between the bacterial dilution series and were independent of the presence of the beer matrix and tested beer styles.

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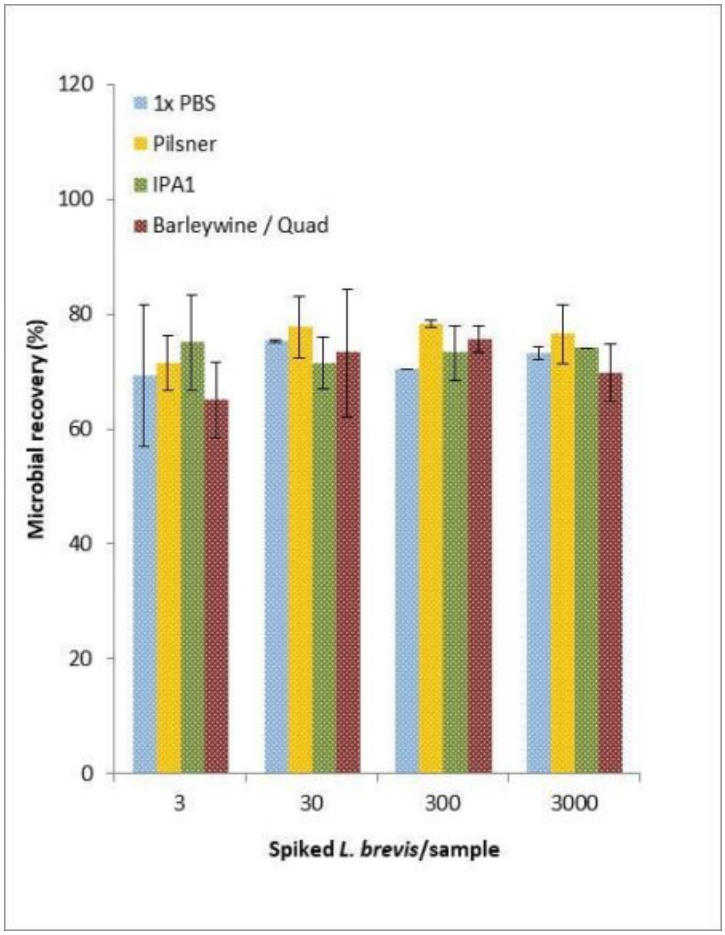

Figure 7. Microbiological recovery rates after sample pre-treatment of beer. 1× PBS (pH 7.4) (**Blue**), a Pilsner (**Yellow**), IPA1 (**Green**) and a Barleywine / Quad (**Red**) were spiked with a 10-fold dilution series of *L. brevis* (3, 30, 300 and 3000 CFU/tested sample) and pre-filtered using 5µm syringe filterdiscs before enzymatic treatments with Proteinase-K and Amylase for 30 min at 37˚C in a background of 0.25×PBS pH7.4 and additional centrifugation at 10000×*RCF*. Samples were analyzed for microbial counts with and without sample pre-treatment using the *Sieve*-ID viable count kit. All samples were tested in duplicate.

2.6 Sample preparation methods for chemical analytes

Sample preparation for the pesticide and heavy metal bio-recognition assays, developed in Task 3.2 and 3.3, is designed as a simple and easy-to-use protocol. The antibody-based bio-recognition assays allow for the direct detection of targets in liquid-based h-ALO defined matrices (aquaponics water, beer, milk, and honey), without the need for extensive or complex extraction procedures. The only requirement to overcome matrix interferences is a simple sample dilution in PBS-TB (for pesticides) or HBS-TB (for heavy metals and pesticides). Four sample preparation protocols have been developed and tested for chemical analytes using the benchmark technologies. For aquaponics water and beer a 10-fold sample dilution in buffer (PBS-TB or HBS-TB) is sufficient for the measurement of chemical analytes. For honey a A 50 to 100-fold sample dilution in (PBS-TB or HBS-TB), depending on the chemical analyte and botanical origin of the honey, is sufficient. For milk a 10-fold sample dilution in (PBS-TB) is sufficient for measuring all targeted antiparasitic drugs, however, this results in poor sensitivities for moxidectin. Since moxidectin is highly lipophilic, the detection in milk requires an additional sample preparation step for detection at EU MRLs, consisting of a fast acetonitrile milk (1:1, v/v) extraction combined with a 5-fold dilution in PBS-EB buffer. A selection of results aquired by simple one step dilutions are shown in Figures 8 and 9.

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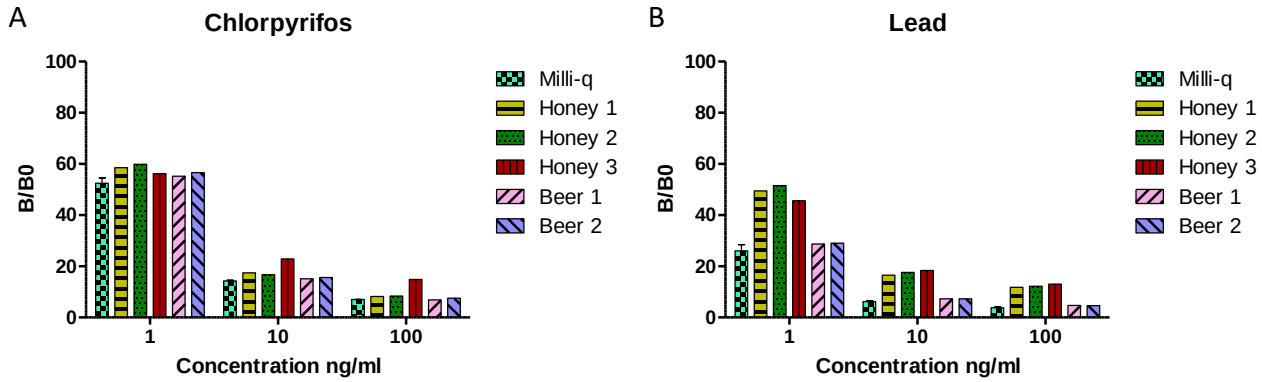

Figure 8. Inhibition profiles after sample dilution (beer 10-fold, honey 100-fold) for the pesticide chlorpyrifos (**A**) and the heavy metal lead (**B**) in MilliQ water (control) and in 3 types of honey and 2 types of beer after.

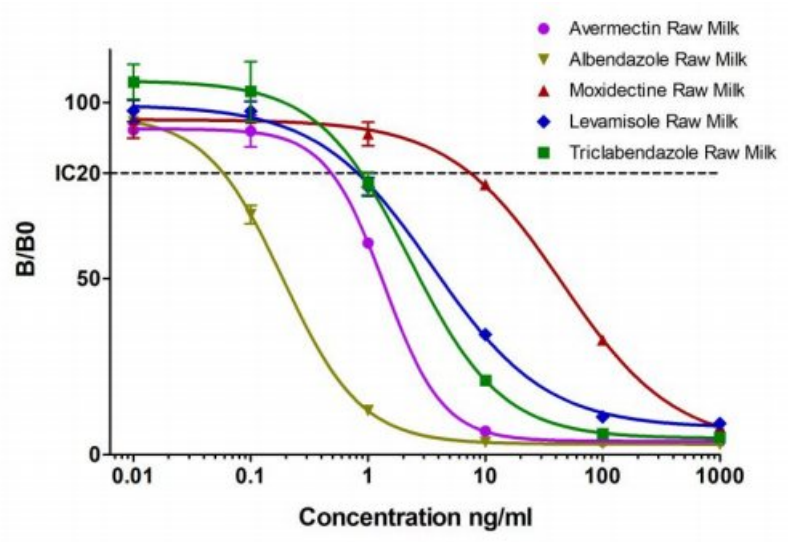

Figure 9. Dose-respponse curves for antiparasitics in raw milk after sample preparation (10-fold dilution in PBS-TB).

2.7 Heavy metal filter compatibility

Loss of analytes due to non-specific binding to surfaces of vials, plates or filters is a well-known problem in analytics. Therefore the loss of mercury and lead, dissolved in Milli-Q or aquaponics water, during filtration through 10µm Acrodisc PSF Versapor filters (25 mm diameter) was determined. Milli-Q water and aquaponics water were spiked with 5, 10 or 100 ppb (µg/L) of lead or mercury. Prior to filtration, samples of 100 µl were taken and diluted with 4.9 ml of 4.2% HNO_3. 2 ml of this sample was filtered, and 100 µl of the filtrate was diluted in 4.9 ml 4.2% HNO_3. The heavy metal concentrations in the samples were determined with inductively coupled plasma-mass-spectrometry (ICP-MS).

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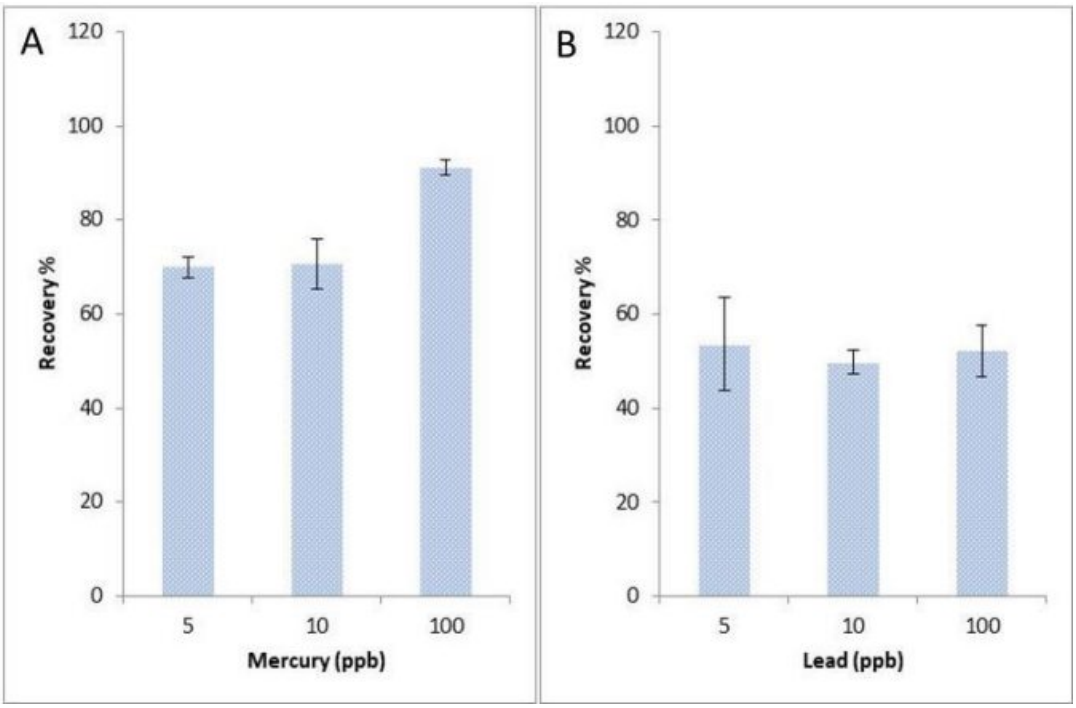

Figure 10. Recovery of (**A**) mercury and (**B**) lead dissolved in Milli-Q water after filtration through 10µm Acrodisc PSF Versapor filters (25 mm diameter). All samples were tested in triplicate.

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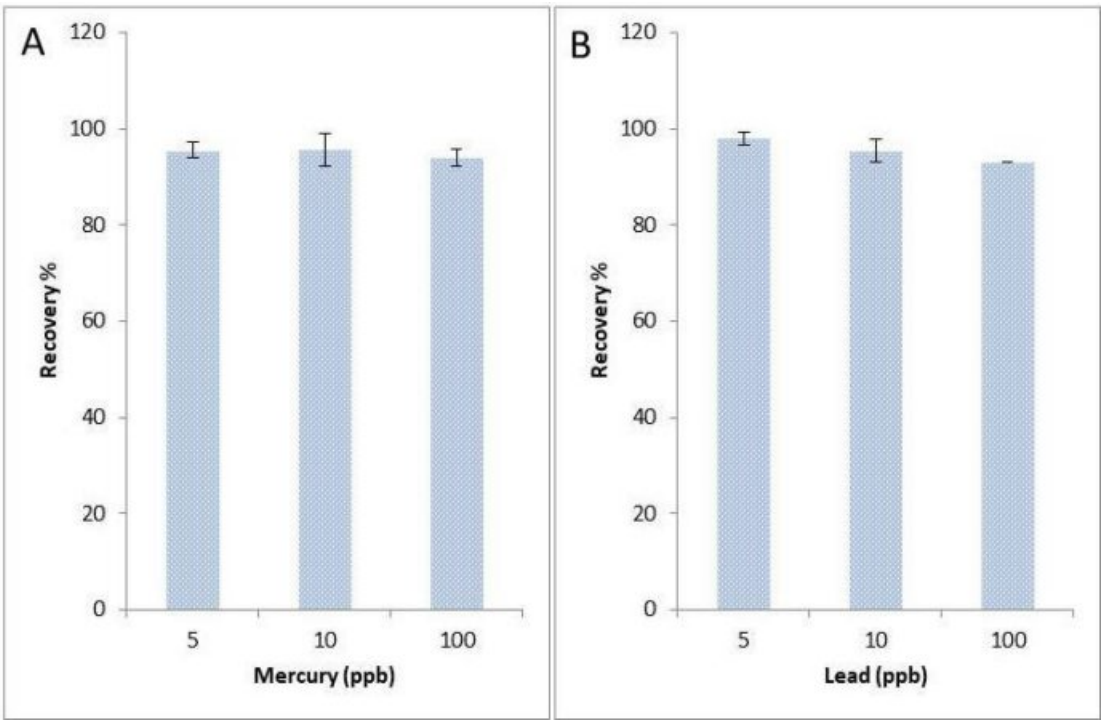

Figure 11. Recovery of (**A**) mercury and (**B**) lead dissolved in aquaponics water after filtration through 10µm Acrodisc PSF Versapor filters (25 mm diameter). All samples were tested in triplicate.

When dissolved in Milli-Q, both mercury and lead bind to the filter can lead to a loss of up to 30% for mercury and 50% for lead (Figure 10). In aquaponics water, almost no loss of mercury and lead is observed, with recovery rates of >93% (Figure 11). As aquaponics water is rich in a wide variety of minerals that can also interact with the filter material, it is likely that the abundantly present minerals block the active binding places in the filter, herewith reducing the level of binding of mercury and lead due to competition. Therefore, no serious problems are expected for the filtration of aquaponics water.

2.8 Pesticides filter compatibility

The absorption of pesticides from liquid-based samples onto materials used in filters, as a conesqunce of their polarity, is a known problem. Therefore, the compatibility of the 10µm Acrodisc PSF Versapor filters (25 mm diameter) with a relevant selection of targeted pesticides was tested. Liquid samples, aquaponics water, diluted honey, and Milli-Q water were multi-fortified with pesticides at a concentration of 100 ppb. As described previously, the samples were simply diluted 10-fold (aquaponis) or 100-fold (honey) in PBST-TB buffer. The Milli-Q water samples were diluted correspondingly as dedicated controls for aquaponics water and honey. 1 mL of each sample was passed through an Acrodisc filter. The filtered and unfiltered samples were tested using the benchmark microsphere immunoassays in a multiplex target set-up.

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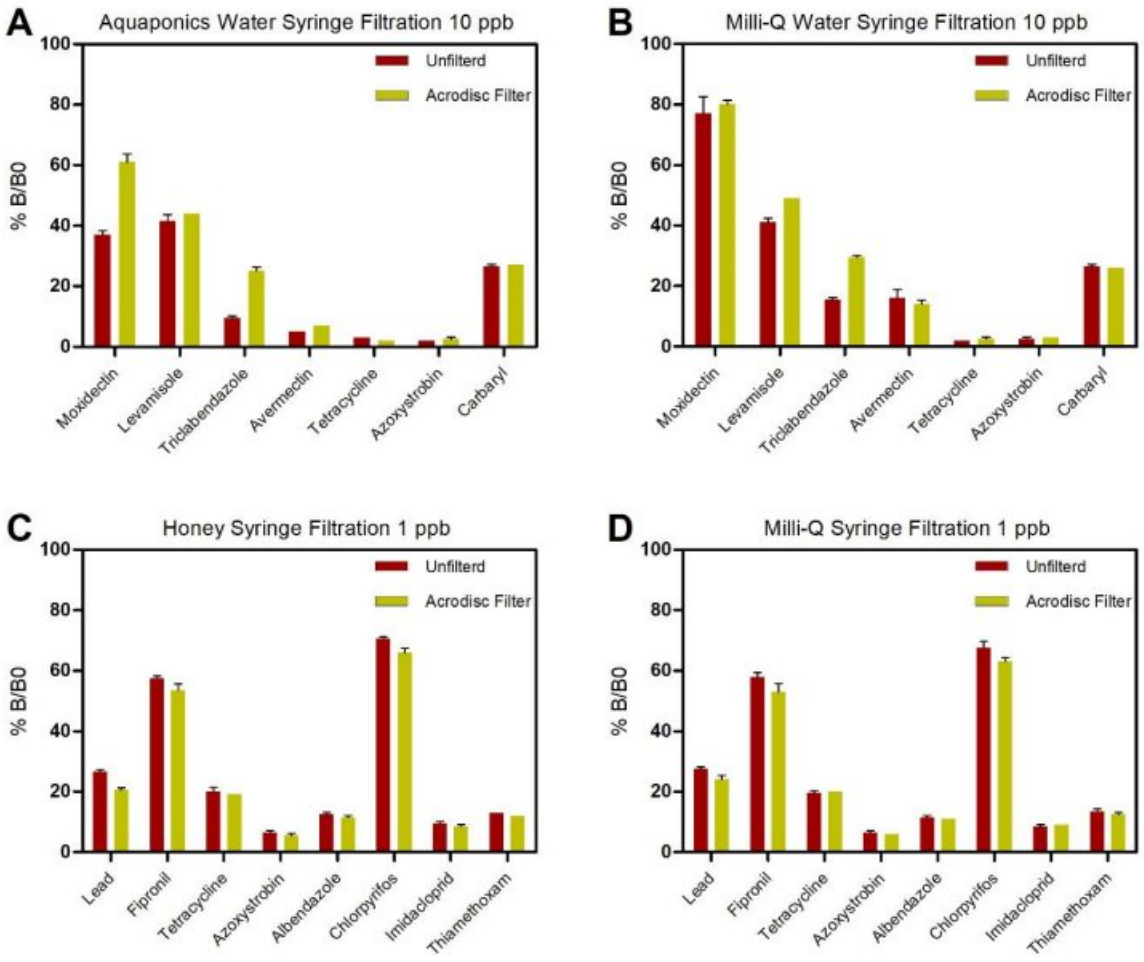

Figure 12. Inhibition profiles of the multi-fortified samples in (**A**) aquaponics water, (**B**)Milli-Q 10-fold dilution, (**C**) honey, and (**D**) Milli-Q 100-fold dilution before (red bar) and after filtration (green bar) through the 10µm Acrodisc PSF Versapor filters (25 mm diameter). All samples were tested in triplicate.

The results show that the antiparasitic pesticides moxidectin and triclabendazole bind to the filter, as shown by the inhibition loss in the corresponding immunoassays (Figure 12). Comparable binding to the filter is only found for triclabendazole in the Milli-Q control sample, but a loss of sensitivity, in general, is observed for moxidectin. No loss of inhibition is observed in the diluted honey and its Milli-Q control sample, indicating that no sticking of targets occurs in honey.

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3 Conclusions

For all four sample types, matrix-specific interferences for microsieve filtration and subsequent analyte collection were identified, and matrix clean-up procedures were successfully developed. The developed sample preparation strategies for microbial analyte detection increased the filterability of the four sample types over the *Sieve*-ID® microsieve membranes. However, only the sample preparation strategies for two of the four matrices were compatible with, both, the microbial and the chemical analyte extraction and recovery.

A single sample preparation strategy for biological and chemical analytes was developed for the aquaponics waters and honey matrices. The developed sample preparation strategy for aquaponic water consists of a simple, easy-to-use syringe filtration step using a filterdisc with 10µm pores. The sample matrix did not interfere with the microbal recovery rates for aquaponics water, which were >87% of all the microbes for all tested samples. The sample preparation strategy was compatible with the chemical analytes. Although in Milli-Q, lead (50%) and mercury (30%) did absorb to the syringe filterdiscs, with recovery rates of >93% in aquaponics water, almost no loss of mercury and lead was observed. Aquaponics water is rich in minerals that may interact with the filter material and compete for binding places with mercury or lead, resulting in minimal analyte absorption. When multi-fortified in aquaponics water, the pesticides moxidectin and triclabendazole bind to the filter, leading to a ±50% loss of inhibition in the immunoassay, but are neverthelss still detectable. No interaction with the syringe filterdiscs was observed for all other tested pesticides.

The developed sample preparation strategy for honey consists of a simple, easy-to-use syringe filtration step using a filterdisc with 5µm pores after the honey has been dissolved in pre-heated 1× PBS (pH7.4). The sample matrix did not interfere with the microbial recovery rates for honey, which were >64% for the spiked *B. cereus* bacteria. Although around 35% of all the *B. cereus* bacteria were lost during sample preparation, this loss in microbial recovery rate was more than compensated by the 10-fold higher filtration volumes achieved upon the selected sample preparation strategy. *Sieve*-ID® microsieve membranes with 3.0µm pores were required for acceptable honey processing, meaning that *B. cereus* capture in the h-ALO sample cartridge relies on surface-modified *Sieve*-ID® microsieve membranes. The sample preparation strategy was also compatible with the chemical analytes; no absorption to the syringe filterdisc was observed when multi-fortified honey was tested.

Different sample preparation strategies were required for analyte detection in raw milk and beer: one for the biological analytes and one for the non-biological analytes. The microbial sample pre-treatment of raw milk consists of centrifugation + chemical/enzymatic treatment + syringe pre-filtration. During the centrifugation step, the non-biological analytes were discarded together with the supernatant, making this sample preparation strategy unsuitable for the chemical analytes. The microbial recovery rates were between 8.5 and 31% and varied significantly between milk samples and the farms of origin. Even though the microbial cell recoveries were relatively low, the increased filtration volumes of milk are more than sufficient to compensate for the loss of microbial recovery. *Sieve*-ID® microsieve membranes with 3.0µm pores were required for proper raw milk processing, meaning that *Salmonella spp.* capture in the h-ALO sample cartridge relies on surface-modified *Sieve*-ID® microsieve membranes. For the chemical analytes, a 10-fold sample dilution in buffer is sufficient to measure most antiparasitic drugs. Because moxidectin is highly lipophilic, the detection in raw milk requires an extra extraction step, consisting of a fast acetonitrile milk (1:1, v/v) extraction combined with a 5-fold dilution in PBS-EP. The microbial sample pre-treatment of beer consists of a syringe pre-filtration (5µm) step combined with a Proteinase-K and Amylase enzymatic treatment. A centrifugation step of 10000×*RCF* can improve the filtration for some of the beer samples/styles, but improvements were not consistent for all beers and probably needs further beer-specific optimization. All beers showed microbial recovery rates between 65% and 78% upon sample preparation. The microbial recovery rates did not differ between the bacterial dilution series and were independent of the presence of

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the beer matrix and tested beer styles. For the chemical analytes, a 10-fold sample dilution in buffer PBS-EP pH 7.4 was sufficient for measuring chemical analytes.

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