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Food Microfluidics: science, technology and creativity making food analysis safer, faster and easier
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Lights and shadows on *Food Microfluidics*

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These insights attempt to share with the community the lights and shadows of one emerging and exciting topic, *Food Microfluidics*, defined as the Microfluidic technology for food analysis and diagnosis in important areas such as food safety and quality. The reader is invited to question non-easy interrogations such as why *Food Microfluidics*, what is the next step and what could we do with the available technology. This article invites to food analysts to be seduced by this technology and then to take an interesting trip departing from the main gained achievements, have a look of the crossing bridges over the *Food Microfluidic* challenges or have a look of available technology to start. Finally, this trip is arriving to a privileged place to gaze the horizons. A wonderful landscape –full of inspiration– for *Food Microfluidics* is anticipated.

These insights have also been written wishing to give improved conceptual and realistic solutions for food analysis, with the additional hope to attract the community with exciting technology, in order to get novel and unexpected achievements in this field.

1- Microfluidics and Food Analysis: *Food Microfluidics*

Excellent literature gives support to this central question: why microfluidics for food analysis? On the one hand, in 2006, *Nature* published an excellent collection of papers devoted to lab-on-a-chip (LOC) technology where G. Whitesides clearly defined *Microfluidics* as the science and technology of systems that processes or manipulates small amounts of fluids (10^{-9} to 10^{-18} L), using channels measuring from tens to hundreds of micrometers.[1] It has implied new drawbacks and new opportunities for all scientific communities since *Microfluidics* exploits both its most obvious characteristic — small size — until and less obvious characteristics of fluids in microchannels offering new capabilities in the control of concentrations of molecules in space and time. These new capabilities (mostly based on the omnipresence of the laminar flow and on the important role of diffusion in microscale) have opened avenues for analytical chemists, among others, to create novel tools for solving emerged and traditional problems[2].

On the other hand, Hamburg´s editorial in *Science* in 2011, states the relevance of ensuring safety and quality of foods.[3] Indeed, one of the most important goals of food analysis is to ensure food safety. To meet this goal, food laboratories have been advised to exchange their classical procedures for modern analytical techniques that allow them to give an adequate answer to this global demand. Consequently, food analysis is, nowadays, one of the most important topics in our society´s concerns as currently, there is also a general trend in food science to link food and health. Thus, food is considered today not only a source of energy but also an affordable way to prevent future diseases[4].

However, the maturity of a certain technology is normally judged by its real life application. In this sense, despite the enormous popularity of microfluidic technology in the scientific literature, their real-life application extent has been quite limited so far in some fields. This is the case of Microfluidics for food analysis [5-8],–termed by us here as *Food Microfluidics*.

The main question is why the food sector cannot be benefited from the advantages of Microfluidics or LOC technology? Why not if in food safety (does this sample contain a pesticide, toxin, foodborne pathogen?) or even in food quality (Is this olive oil of quality?) could we meet a faster, simpler and probably cheaper response to the real-world demand?

The general challenges in Food Analysis have been featured[4] Technological challenges such as the miniaturization of analytical systems with especial attention to

Microfluidics and, those related to the detection of important molecules and bio-systems such as detection of food borne pathogens (bacterias, viruses), toxins, allergens as well as the emerging toxics including nanomaterials (NMs) have been proposed. Finally, the implementation of green analytical chemistry also in food analysis has also been identified as important challenge[9]

Interestingly, in this landscape of challenges in Food Analysis, *Food Microfluidics* constitutes a challenge by itself. This identification makes *Food Microfluidics* a valuable tool for Food Analysis.

Indeed, Microfluidics allow us to analyse samples in a very short time, using extremely low sample and reagent volumes and generating inconsiderable residues (being a clear green analytical chemistry approach) with high capacity for multiplexing analysis. Also, microfluidic devices are potentially very attractive in food analysis because of their possible additional advantages such as cheap, portable and (fully automatized and integrated) systems used on-site by anyone *in the field* and, even disposable. Also, some "unknown" advantages are those in which the *Microfluidics* allows the accurate and easy fluidic manipulation of several fluids simultaneously opening new avenues to perform analysis with creativity. In other words, controlled-driven fluidics allows controlled-driven food chemistry. Since they are unknown, they are consequently underexploited.

In addition remarkably, important problems in food safety and food quality fields could be approached from *Microfluidics* side since they could release not only fast but also reliable solution as it is urgently demanded under these circumstances. In this way, since we are living in one society where more information regarding food quality and safety is demanded[3,4], and a profound impact of these topics on the global health will be take place, an exciting future for these technologies is also expected.

Figure 1 provide information on the number of works published in the period 2000-2013 found though a search in the database ISI of Knowledge using as key words Microfluidics and the names of application fields (clinical & health, environmental and food) (A) and Microfluidics, food analysis and the names of detection analytical techniques (B). In spite of these conceptual advantages of *Food Microfluidics*, only a 1% of the all applications in Food Analysis use Microfluidic technology (results are not shown in **Figure** 1). **Figure 1A** reveals that Microfluidics for food analysis has also been less explored than others such clinical (5-fold lower) and environmental (2-fold lower). Also, **Figure 1A** shows the distribution of publications from 2000 to date and

prediction for 2020 in *Food Microfluidics* starting in the early 2000 and growing up until date with an important increasing the number of articles.

Probably, one of the main reasons to understand this delay in the appearance of works dealing with microfluidics for food analysis and the lower number of published works in comparison with other fields is the inherent complexity of the food samples which requires enhancements of sensitivity and selectivity during sample preparation step and the integration of this step in Microfluidics is still a major technical challenge. The literature in the field has quantitatively converged in this challenge identification[5-8]. Indeed, microfluidic technology has also shown interesting applications for food analysis, although more effort has to be put on the development of multipurpose microfluidic platforms that integrate multiple unit operations for real food sample analysis.

Although LOC technology in Food Analysis has been less explored than others as it was stated before, some outstanding achievements can be identified. Next, the main achievements gained in the field will be identified and briefly discussed in two well-separated sections: separation and detection systems and microfluidic biosensing in *Food Microfluidics*.

A- Target molecule detection and separation in Food Microfluidics

On the one hand, from the beginning, detection has been one of the main challenges of *Microfluidics* since very sensitive techniques are needed as a consequence of the use of ultra-small sample volumes introduced in these systems. Laser Induced Fluorescence (LIF) was the original detection technique applied because of its inherent sensitivity and easy focusing. However, from the early times, electrochemical detection (ED) constituted the most attractive alternative because of its inherent sensitivity and miniaturization without loss of performance and high compatibility with the microfabrication techniques typical in Microfluidics.[10] Specifically speaking in *Food Microfluidics*, ED has been successfully implemented in both amperometry and conductometry approaches. As it is shown in **Figure 1B**, the prominent role of ED in *Food Microfluidics* is nowadays, unquestionable. Indeed it has been widely explored (almost the 50% of all food applications used mostly amperometry and conductometry) in comparison not only with mass spectrometry (MS) (only a 5%) but also with the well-established LIF (17%). In addition to previously stated advantages of ED (inherent miniaturization highly compatible with microtechnologies, high sensitivity and low cost); the large number of electroactive analytes with food significance, the suitability of conductometry for detecting ionic food analytes and the no dependence of

electrochemical responses of sample turbidity justifies the large use of this detection principle in *Food Microfluidics* in comparison to LIF approaches which are expensive, non-miniaturized and need derivatization.

Recently, from one personal reflexion [11] I have said "[…] *electroanalysis is living a true Renaissance. Inherent miniaturization of electrochemistry makes it a unique detection and transduction principle, highly compatible with micro and nanotechnologies. It also implies advantages on portability and further disposability. Another very unique feature linked to electrochemistry is the versatility for "selectivity design" towards the suitable selection of (nano-) (bio-)-materials and by the direct manipulation of the electrical properties. Their remarkably sensitivity and low cost are additional valuable features. However, from my personal perspective, these natural beauties are underexploited in the analysis of food samples not only because of the complexity of food samples but also electrochemistry has traditionally been seen as "a difficult thing*". If besides we add the word "microfluidic" the degree of difficulty could become enormous. We should try to change this perception in order to introduce *Food Microfluidics* in our labs as it will be discussed latter. I am sure that the synergy between electrochemistry and *Microfluidics* is a hot and exciting topic as I have already written in several editorials[12]

On the other hand, while ED has been one of the main achievements in *Food Microfluidics*, microchip electrophoresis (ME) –as example of microfluidic chips (MC)– has been a clear achievement in the separation sciences in LOC technology[13] and, as consequence, it has successfully been transferred to *Food Microfluidics* as well[8,11]. Indeed, the well-established microfabrication of a network of channels using materials of well-known chemistry (mainly glass and polydimetilsyloxane (PDMS)) and the easy possibility of using the electrokinetic phenomena to move fluids, justifies why they have successfully been implemented. In addition, since a very important group of analytes of food significance can be analyzed by capillary electrophoresis (CE) and ME is based on the same principle of conventional CE; their transfer to microchip format has also been widely explored.

The earliest applications in *Food Microfluidics* (reported more than 10 years after the pioneer Manz´s works[2b] in 1992) were focused on the exploration of fast separations and suitable detection routes of prominent analytes with food significance as an example of "proof-of-the-concept" and/or their detection in easy non-ideal samples. In addition, simple microchip geometries and layouts using both, glass and PDMS, coupled to preferred ED route in both, amperometry (end-channel configuration) and conductivity (contactless) formats were explored[8a]

Then, in a second step different strategies to improve the selectivity and sensitivity of the analysis by avoiding and/or making the sample preparation as simple as possible were used: (i) enhancing the peak capacity in order to perform direct injection, (ii) using the microchip platform to measure one target analyte/group of analytes (even with separation of related interferences), (iii) integrating some sample preparation steps such as preconcentration using electrokinetic approach on the microchip platform, and (iv) integrating new analytical tools from nanotechnology in the detection stage. As a consequence of these strategies, new analyte separations of food significance involving DNA probes, biogenic amines, vanilla flavours, and dyes were reported as successfully breaking new barriers in areas of high impact on the market, such as transgenic food analysis, as well as the detection of frauds and toxins. Simple microchip layouts were again the most common designs used, though some sophisticated ones were emerging. In contrast to other application areas, ED continued to be the most common detection route, followed by LIF, though some non-conventional detection routes were also reported, such as chemiluminescence or UV.[8b]

From 2008-to date, basically, single cross ME design has been used for food analysis with ED and LIF being the most common detection principles coupled. In the last four years the main outlines were: (i) the exploration of new analytes such as heavy metals, nitrite, micotoxins, microorganisms and allergens; and interestingly (ii) although sample preparation is still performed off-chip, an important increase in works dealing with complicated food samples has been clearly noticed. Important fields such as authentication of foods, detection of frauds, toxics and allergens were also explored[8c]

B- Microfluidic chemical sensing and biosensing in Food Microfluidics

Microfluidic technology has now become a novel sensing platform where different analytical steps, biological recognition material and suitable transducers can be cleverly integrated yielding a new sensor generation which could be termed as microfluidic (bio-)-sensors. These microfluidic biosensing platforms have integrated part or all the necessary components of a bioassay procedure making use of a network of microchannels and/or bio-reactor chambers usually built in a monolithic platform (MCs) from different materials as glass or polymers. These microfluidic platforms are very suitable for bioassays because in microchannels, the surface area to volume ratio is higher, making the diffusional distances dramatically reduced and producing lower analysis times improving the efficiency of the bio-recognition and transduction reactions. Also, automated procedure can be potentially performed since different steps and fluid movement can be easily controlled, especially with electrokinetic fluidic

motivation, through the adequate control of applied electric fields, or in a more complicated way by the use of pumps, valves and mixers[14]

These microfluidic biosensors for food safety have mainly been developed into immunoassay format focused on the detection of mycotoxin/toxins, food-borne pathogens (bacterias, viruses), drugs and allergens[14-16]

In the case of mycotoxins, which can be found as contaminants in cereals, related products used for feed, beverages as fruits juices and wines, foodstuffs and their products worldwide, are considered an important source of health and economic problems. Reliable assessment of several mycotoxins such as citrinin, ochratoxin A, and zearalenone in rices, fruits and feedstuffs has been approached using microfluidic bio-sensing[14].

A highly significant group of analytes explored using this approach is the food-borne pathogens. Indeed, hundreds of foodborne infection cases occur around the world, and up to one-third of the population in industrialized nations suffers from foodborne illness each year. Regarding pathogens detection in foods, microbiologists have developed over the last decades reliable culture-based techniques. Although these methods are considered to be the “gold standard”, they remain cumbersome and time-consuming. In this way, apart from the Microarray-based technologies, Microfluidics represent an advance in food pathogen testing methods whose main features include miniaturization, ability to parallelize sample processing, and ease of automation. Tolerable levels of these agents are getting more stringent regulations due to the high concern of people for food safety. Bacteria such as *Escherichia coli, Staphylococcus, Shigella, Listeria, Salmonella, Campylobacter, Clostridium* are considered some of the most dangerous food-borne pathogens which have been explored using microfluidic approach since it is necessary their rapid, sensitive and reliable detection[15,16].

Other biological recognition platforms have been reported for detection of food borne pathogens (bacteria genus previously mentioned and viruses such as rotaviruses and calciviruses) mainly based on nucleic acids-based probes[15,16]. They are usually more specific because the epitopes, present on the surface of the cell and recognize by antibodies, are normally found throughout the species. However, they are based on microarrays or highly complicated microfluidic platforms, where amplified nucleic acids sequences derived from pathogens are usually determined in longer time and with large manipulation.

Having said this, in summary **Figure 2** illustrates and identifies the main strengths discussed previously (left panel) as well as the main weakness which constitutes the challenges (right panel) clearly separated by the physical frontier in the field of *Food Microfluidics*. The suitability of electrochemistry for food analytes detection as well as the relevant maturity of ME technology, make them important achievements for microfluidic separation and (bio-)-sensing in *Food Microfluidics.* On the contrary, the complete integration of sample preparation as well as the integration of very sensitive detectors to achieve low detection limits in the small sample volumes remains as one of the most important challenges in *Food Microfluidics*[5-8]. Indeed, because of the complexity of the food samples, in *Food Microfluidics*, selectivity and sensitivity requirements often involve complex sample preparation and/or analyte separation with very sensitive detection schemes. The complete integration of sample preparation in microchip technology is appealing challenge since it requires sophisticated micro-fabrication facilities to develop microstructures for filtering, pre-concentration and clean up and even derivatization to make analyte compatible with the very sensitive detectors required as it was stated above. In addition, real-world interface is another appealing challenge when many processing of food samples is required.

Finally, I think that one important philosophical “hidden” challenge is that Microfluidics is still seen as an expensive, inaccessible and difficult “thing” by some part of food analyst community.

2-What is next? Crossing bridges over *Food Microfluidics* challenges

What is next? The answer to this important question starts taking a look at the **Figure 3**. From my personal perspective, the construction of solid bridges over *Food Microfluidics* challenges needs; on the one hand, not only smart tools from technologies inherently involved into Microfluidics, but those from other technologies– highly compatible in scale– which can additionally offer an improved analytical performance, such as nano and bio-technologies.

Tools from key-technology reservoirs (micro, nano and bio-technologies) need to be ”found, pump and flow” into the microchannels. Under “laminar conditions and diffusion in action”, solutions will be given. Indeed, they should come from the interfacial work between these target technologies, and realising commercialization at the end of the channel before going to the real world, in *Food Microfluidics* as is illustrated in **Figure 3**.

On the other hand, from an analytical point of view, to deal with the complexity of the food samples, two strategies could be approached involving the technologies stated above: (i) those including integration of sample preparation on Microfluidics (that means technical facilities to microfabricate the elements for filtering, pre-concentration, clean-up, and even derivatization); and (ii) those trying to avoid or to make sample preparation as simple as possible using smart molecules (biomolecules) and NMs with added selectivity and sensitivity, even using suitable chemistry functionalization.

Next, we will discuss in brief the main outlines of the previously stated solutions

Researching microtechnologies (main channel)

Firstly, micro technologies (creation of physical and sophisticated structures) and the inherent features of *Microfluidics* (omnipresence of laminar flow and lateral diffusion) offer unique and very creative opportunities for the integration of filtering, extraction and preconcentration steps. [17]

Due to the typical small dimensions in microstructures, particles/beads can cause serious operational problems, providing sites for nucleation or blockage being filtration an important step to be integrated. Two approaches have been proposed: structurally-based filters (filtering and retention by integrated flow restrictions and controlled by manufacturing process) and diffusion based filtration (filtering by diffusion in laminar flow) where the transport of material only occurs by diffusion due to the omnipresence laminar flow in microfluidic systems.

Extraction approaches (liquid-liquid and solid-liquid) have been another important challenge in the integration of microfluidic systems. The high surface-to-volume ratio and the short diffusion distances, typically within microfluidic environments, combined with laminar flow conditions, offer the possibility of performing liquid-liquid extraction within microchannels without shaking. Packing microchannels with stationary phase or with continuous porous beads/layer *in situ* formed from polymerisation of organic monomers has been used as solid-phase extraction and preconcentration.

However, while these achievements briefly commented have not been placed into *Food Microfluidics*; on the contrary, electrokinetic flow-driven pre-concentration approach for achieving high sensitivity in microchip format have been explored for analysis of dyes. [18] The microchip consisted of three parallel channels. The first and the second were used for the field-amplified sample stacking and the subsequent field-amplified sample injection steps, while the third was reserved for the micellar electrokinetic chromatography with ED.

While flow focusing approaches are easily applied when electrokinetics is used; however, in general, all previous approaches potentially applied to the food analysis, require microfabrication and facilities (very often clean-room ones) which frequently are not available in common labs. If we want to export the microfluidic technology to the food applications, is necessary to make microfluidic fabrication more available for the community in terms of accessibility and costs. In this context, the exchange of ideas, between food analysts and microfabrication scientists for design of chips for tailored applications is of paramount significance.

Besides of the PDMS technology (which has been explored in *Food Microfluidics*[8]), the development of novel micro-technologies which do not require clean room facilities could be a valuable alternative. One relevant example of these micro-technologies, where *Food Microfluidics* could meet relevant application is the Microfluidic paper-based analytical devices (micro-PADs). Micro-PADs are a new platform for analytical purposes, which combine some of the capabilities of the conventional microfluidic devices with the simplicity of diagnostic strip tests.[19] These systems are made by pattern hydrophilic-hydrophobic contrast on a sheet of paper in order to create micron-scale capillary channels on paper (the hydrophilic channels –paper- are surrounded by hydrophobic barriers).They can provide analysis in a more rapid, less expensive, easy to use, portable and more multiplexed way than current analysis being one of their main features only small volume of fluid and little or external supporting equipment or power, since fluid movement in micro-PADs is largely controlled by capillarity and evaporation. Paper-based microfluidic devices are still at an early stage of development and they present some important limitations, which are related to the material properties of paper, fabrication techniques and detection methods incorporated to the devices. Nowadays, these systems are almost fully dedicated to the biochemical analysis, since their special features are particularly relevant for point-of-care (POC) of clinically relevant bio-analysis. However, I see its introduction in *Food Microfluidics* –mainly in food safety (i.e. for food-pathogens *in situ* detection in developing countries)– as an interesting and realistic alternative to be explored.

Researching nanotechnologies (lateral left channel)

Secondly, in my opinion, one extremely important scenario for further development of *Food Microfluidics* is *looking at* and *looking for* nanotechnologies which are pumping from another reservoir flowing into the left channel in **Figure 3**. Micro and nanotechnologies are exciting interfaces –highly compatible in scale– full of possibilities, which can improve the sample preparation simplifying the overall process

since they could give us the selectivity and sensitivity required. While it has stated that *Microfluidics* meets NMs[20], here the idea is the other way around that *Food Microfluidics* need to meet NMs.

Indeed, *researching* nanotecnologies with attention we can find –as a natural step ahead–, conceptual solutions such as combine the "maturity products" (for example MC or specifically ME with ED) with NMs into a novel marriage to enhance sensitivity and selectivity. In my opinion, here we are *looking at* the novel generation of *Microfluidics* and a realistic alternative for *Food Microfluidics*.

For example, when NMs are used as electrochemical detectors of MCs, NMs can significantly improve the analytical performance of chips[21].The scale of a typical NM is compatible with the scale of a typical MC, and the NMs can offer lower detection potentials which improve selectivity, high currents because of their large surface areas, thereby enabling large-scale redox conversion, which increases the analytical sensitivity, resists passivation, and yields very good performance reproducibility. Therefore, the MC-NMs coupling is very pertinent!

Let´s see two examples involving NMs in the detection stage in *Food Microfluidics.* Ultra-fast microfluidic separations coupled to carbon nanotubes (CNT)-based detectors have demonstrated enhanced sensitivity in comparison with those obtained without CNT for a wide of analyte groups of food significance (dietary antioxidants, water-soluble vitamins, vanilla flavours and isoflavones) in representative complex food samples. This approach has allowed solving specific challenges during the analysis such as the direct detection of analytes in the samples avoiding the integration of complex pre-concentration steps on these microdevices[22,23]. Another interesting example is the coupling of copper nanowires to MC which exhibits electrocatalysis towards carbohydrates becoming a "selective detector" with expected enhanced sensitivity. This coupling has been pioneer demonstrated an impress performance[24] and then, it has been explored for the fast and reliable analysis of monosaccharides in honey samples, as well. [25] NM "added the wished/wanted selectivity" and it is an illustrative example about how micro and nanotechnologies *strategically driven* solve a problem.

Apart from the exploration of well-established NMs for *Food Microfluidics* to improve selectivity and sensitivity, the development of novel and easy nanotechnologies is a crucial issue ahead for further developments, expecting in a relative short period of time. One selected example is illustrated in the **Figure 4** where CNTs are press-transferred on polymethilmethacrylate (PMMA) substrates for electrochemical

microfluidic sensing.[26] This is a novel alternative with clear advantages such as (i) CNTs are the exclusive transducer, (ii) these electrodes can be fabricated from commercial sources using a simple protocol which could be afforded in any laboratory, and (iii) they are well-matched with mass-production, disposability and other NMs and/or biological material. These pioneering nano-scaled detectors coupled to MCs have been proposed for fast and reliable qualitative and quantitative assessment of class-isoflavones with excellent results.[27]

It is important to point out that not only NMs meet *Microfluidics* in the detection step but they can be also potentially incorporated in other steps in *Food Microfluidics*. Indeed, the high specific surface and relatively easy functionalization as well as the catalytic properties exhibited by them are very valuable to perform novel separations, pre-concentrations and related analytical operations[28]. Especial attention should be made to magnetic NMs since they offer simplicity (easy manipulation by using external magnetic fields) and versatility in the microscale. Also, the exploration of novel NMs with well-documented features stated before which could give improvement in selectivity, sensitivity and reproducibility (i.e. grephene), hybrid NMs (carbon NMs and nanoparticles) specially combining molecular recognition towards the construction of novel smart (sensitive and selective) detectors is another clear step ahead in the immediate horizon for a success of *Food Microfluidics*.

Researching biotechnologies (lateral right channel)

Thirdly, another clear elegant alternative to avoid the complex approach of the sample preparation integrated on chip is the development of novel strategies for microfluidic bio-sensing (biotechnology reservoir) as it is also illustrated in **Figure 3.**

The creative use of bio-molecules with high selectivity taking the unique advantages of *Food Microfluidics* (extremely low sample consumption, fast analysis times and environmental friendly) is one of most elegant and efficient strategies to achieve the required selectivity and sensitivity in *Food Microfluidics* without the need of the integration of complex sample preparation steps and avoiding, consequently, microfabrication.

In contrast with other diagnosis fields where the biological reagents are used frequently and dramatically improve the selective analysis; in food analysis, the use of biomolecules is less explored becoming sample preparation mostly needed. Consequently, the development and commercialization of non-expensive and novel bio-molecules for food analysis is another important issue. Without any question, this

development in conjunction with the development of micro and nano-technologies will allow a solid success in the microfluidic sensing and biosensing developments.

As selected food safety example, a novel LOC strategy integrating an electrokinetic magnetic beads-based electrochemical immunoassay has been creatively proposed for reliable control of permitted levels of the micotoxin Zearalenone in infant foods.[29] **Figure 5** illustrates the creative use of the simple channels layout of double-T microchip to perform sequentially the immunointeraction and enzymatic reaction by applying a sequence of electric fields suitably connected to the reservoirs for driving the fluidics at different chambers in order to perform the different reactions. This approach avoids classical sample preparation, becoming a truly LOC for fast and reliable food diagnosis "making baby food safer" as it was highlighted in *Chemical World News*.

Finally, in the **Figure 3** *smart* (bio-nano-) detectors (sensitive and selective) are well placed at the end of the merged channels just before commercialization. This constitutes by itself one of the most important expected achievements.

In spite of all said, important advances should come from the industry. The improvement of commercialization of easy microfluidic chips "ready-to-use" needs to be driving in the foreseeable future. This is the unique exit to succeed in the real world, as it is shown in **Figure 3.**

Although several companies have entered in the agro-food sector as it was revised[6], the community cannot await one specific "commercialized product" for each specific solution! In addition, we must demonstrate the microfluidic advantages to the community in order to attract the market, looking for the market or creating the market. One possible solution is to improve the commercialization of microfluidic products (MCs) potentially containing as many as possible opportunities to solve common problems. These MCs need to be "easy to buy" because they are "easy to use" and cheap: lowering the cost and making them even disposables.

One representative example recently explored in *Food Microfluidics*, has been the commercialization of disposable MCs made in a hybrid material polymer/glass like SU-8/Pyrex with integrated electrochemical detectors. This approach cleverly combines the advantages of low cost and easy fabrication of SU-8 with the high performance of glass for chemical analysis. Recently, these chips have demonstrated to be a powerful analytical tool for the determination of phenolic compounds in complex food samples.[30*a,b*]

3- Microfluidic chips for *Food Microfluidics*

While solutions stated before are arriving, we "*cannot be waiting settled*". The immediate success of *Food Microfluidics* is in our inspiration & creativity, our faith in them and our perseverance to demonstrate that *Food Microfluidics* can replace the traditional approaches or, in other words, the *big achievements could be made just using small tools*. Indeed, from a conceptual and holistic point of view, the overall solution becomes clear: creativity needs to replace facilities as long as it could be possible. While facilities are limited, creativity remains unlimited. Creativity is the "unused/waste" technological reservoir. A good analytical chemist is that who knows and uses the most suitable analytical tool to solve the analytical problem. Microfluidics is a unique technology full of possibilities for fast, reliable multiplexing and non-specialized analysis.

While in Food Analysis, to meet the targets, food laboratories have been advised to modify their classical procedures for modern analytical techniques that allow them to give an adequate answer [4]; in order to see a success of *Food Microfluidics*, food analysts are invited to modify their available procedures (very often from modern and sophisticated analytical techniques to solve common tasks) to microfluidic solutions with enough analytical potency to give the required answer (*fitness-to-purpose*)

In this way, and although, sample preparation will be still performed *off-chip*; two realistic key strategies become for a rapid success of *Food Microfluidics*: (i) sample screening methods and (ii) the downsizing of the traditional methods (sometimes unnecessary), which requires long analysis times, high consuming and tedious procedures. As consequence, the success of *Food Microfluidics* passes through the technology, replacing the traditional approaches. With these purposes we have already a valuable commercial tool: MCs

In this context, one proposed route to start work in the field immediately is illustrated in **Figure 6** consisting of the following steps: (i) Choosing the application with food significance (and realistic possibilities to be solved by the available technology), (ii) evaluating analytical performance (with especial attention to analysis of real samples although sample preparation could be performed using an *off-chip* approach to demonstrate the potency of the technology), (iii) integrating methodological calibration as required control for reliable and quantitative analysis, (iv) moving to the parallelization and multiplex of the analysis (with integrated calibration, if analytical performance of the food system is good enough), and finally (v) to explore prototyping and commercialization for portable, easy "in field" non-specialized analysis. Some

selected works developed by us in food safety and food quality fields –following the proposed route in **Figure 6–**, will be briefly discussed.

Sample screening methods are approaches in which the positive samples are identified to clearly reduce the time and the cost of the confirmatory methods. Since some tedious sample preparation schemes are often mandatory, and the confirmation techniques are usually sophisticated and expensive, sample screening methods become very useful to obtain a fast response concerning the composition of the sample. The solutions based on the binary response YES/NO constitute inherently one of the main microfluidic markets in the agro-food sector. The development of screening multiplexed MCs in the common labs or *in field* analysis could be a realistic solution if we are able to be creative in the way "fitness-to-purpose".

In this *Food Microfluidics* "just born" for fast and reliable sample screening, simplification of the calibration process will conducts to perform an easy calibration by a non-specializer and even self-calibration for future *in field* analysis. To this end, a methodological innovation integrating calibration and analysis of target food molecules has been proposed using the commercial available technology. Indeed, the strategy consisted in sequentially using both reservoirs (the usually unused sample waste reservoir for calibration and the other one for the analysis). This strategy has improved the analytical performance and it constitutes an interesting added value for food field determinations. For example, the integrated calibration and determination of water-soluble vitamins consumed 350 s in the overall protocol (employing 130 s in calibration plus 130 s in analysis).[31a,b] Remarkably, this approach avoided also the typical four-parameter logistic curve fit obtained during immunoassays for micotoxin determination, which is a highly time-consuming and laborious procedure.[32] **Figure 7** illustrate the fast flight of MC over cereal lands seeking hidden zearalenone mycotoxin when calibration (in blue) and analysis (in red) is sequentially measured using both reservoirs.

Also, *smart* well-designed separations on Microfluidics working as truly sample screening & analyte confirmation approach offer us attractive possibilities for food solutions. One selected example in the food quality sector is the fast separation of the finger-print markers of Vanilla planifolia on microfluidic-electrochemistry chip for assessment of possible frauds. The "problem" was solved just in one single analysis under 250 s because -as it is illustrated in **Figure 8-** the migration order was strategically connected with sequential sample screening (detection of syntethic marker ethyl vanillin (EVA) which allowed the confirmation of non-natural origin) and analyte confirmation (finger-print markers detection of vanillin (VAN) p-hydroxybenzaldehyde

(PHB), vanillic acid (VANA) and p-hydroxybenzoic acid (p-PHBA) which allowed the confirmation of flavour authenticity).[33]

Another well-developed example in *Food Microfluidics* has been the analysis of antioxidants and evaluation of their antioxidant activity, which has generated an important piece of work, recently revised.[34] In this field, the third example to be shown is regarding the creative and selective microfluidic platforms to integrate and simplify on a microscale the traditional methods for complex natural antioxidants determination. In this example, two approaches (class-selective electrochemical index determination and individual antioxidant determination) are proposed for the analysis of nine antioxidants (phenolic acids and flavonoids) in food samples allowing a fast and reliable determination of the main antioxidant classes (flavonoids and phenolic acids) in less than 100 s and an impressive separation of nine antioxidants in less than 250 s.[35] Partially, this approach has also been successfully transferred to the "easy-to-use" SU-8/Pyrex microchips.[30a] The reliability of ME-ED approach was demonstrated towards the high agreement between the total phenolics obtained using microchip approach with those obtained by the well-established classical HPLC-DAD approach. These results suggested that the microchip approach is a reliable method for fast assessment of antioxidants constituting a very good alternative to the long analysis times and the using of toxic solvents required in HPLC. However, in spite of these beauties reached, the *tout of force* was this "simplified product" gave enough information for solving the problem.

4-Horizons

Although in the early times, the development of micro-TAS concept was not born for food applications, the potency of Microfluidics, for fast and reliable diagnosis in extremely important sectors of our society such as food safety and food quality, is enormous. This is a clear example in which the apparition of one technology full of promises is able to generate multiple benefits giving additional values to those gained.

It is crucial to continue in the *growing up* since, in general, *Food Microfluidics* is living still their *adolescence* and one important piece of the scientific community is not under microfluidic seduction, being still seen as a "difficult and expensive thing" only available by a few privileged communities. But, fortunately, like in real life, the adolescence is plenty of dreams and possibilities. The success of *Food Microfluidics* strongly depends on our creativity, since the full integration of sample preparation on chips probably will not be the solution in the near future. Total integration and world-to-chip interfacing are considered the major challenges, particularly in high-throughput applications, requiring frequent sample changes, such as continuous *on-line* process monitoring.

Bio and nanotechnologies are identified as one of the most important key-reservoirs where "*look for & find"* a novel microfluidic solutions for food applications which need to be pump and flow. Easier and less expensive commercialization of biomolecules will be a very valuable help to develop novel microfluidic biosensors with high capabilities for *Food Microfluidics* in the food safety sector. NMs could also improve the chemical sensing by themselves since they improve analysis performance and opens new avenues for future implementation of applications in the field of food analysis. These novel smart materials cleverly combined with biological molecules and miniaturized sensitive detectors draw an extraordinary landscape for expected and non-expected synergies in *Food Microfluidics*. Selected examples, previously exposed, have also illustrated this.

While we are awaiting advances and extension in use of microfabrications and more commercialization, although sample preparation will be performed *off-chip*, several "food things" could be approached from microfluidic side with creativity (especially in those requiring low sample preparation, sample screening methods and downsizing conventional approaches), as it was discussed in the text. Those needs which require fast and reliable solutions are convoked to be solved from the exciting side of *Food Microfluidics*. Both food safety and food quality are typical examples, since our society demands rapidly more and more information (food safety, nutrients, origin denomination and detections of quality markers in natural products to distinguish those

manufactured…). It is important to keep in mind that both food safety and quality have a profound impact on the field of health as well. As consequence of it, breaking and expanding frontiers is also expected and I foresee more impact of *Food Microfluidics* in the health sector imminently.

In addition, it is important that the food community is opened and ready to use "mature microfluidic products". For this reason, it is crucial to improve the commercialization of *Food Microfluidics* (i.e. simple and versatile microchips to perform different similar analysis) which can operate replacing the conventional methods. The fast analysis times even performed into multiplexed forms, the well-demonstrated reliability and the enormous potential for analysis in field are very unique advantages from this technology not easy to be found in others, although sample preparation will be carried out *off-chip* yet. It is just the moment of replacing "old times by new ones", it is time for re-decorating our labs.

Rigorous analytical evaluation of these LOC approaches is mandatory to demonstrate that *Food Microfluidics* offer not only faster but also reliable solutions in the sector, and as consequence of this, analytical chemistry plays a relevant role in the success of the further developments.

Finally, it is extremely necessary to point out again that industry plays also an important role since the full-solved commercialized applications could be delivered from this side. During my best dreams, I can foresee for the future "wine chips" or "toxin chips" just to mention two important markets.

After reading these pages, I conclude with my personal response to the first question stated and the beginning of this insight, *Food Microfluidics:* yes or not? My "expected" response is yes, sure. Like in real life, important drawbacks remain every sunset but clear opportunities are easily seen each sunrise. This is *Food Microfluidics*: an exciting dynamic landscape of sunrises and sunsets; drawbacks at the night become opportunities in the early morning.

Food Microfluidics (integrating bio-nano-technologies): an unlimited scenario with lights and shadows where dreams become reality.

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677 **CAPTION OF FIGURES**

678 **Figure 1**. Pie charts showing the percentage of publications in environmental, food and
679 clinical & health fields in microfluidics as well as distribution of publications in food
680 microfluidics from 2000 to date and prediction for 2020 **(A).** Detection methods
681 employed in food microfluidics (exclusively articles) using contact-less conductivity
682 (CCD), mass spectrometry (MS), chemiluminiscence (CL), laser-induced fluorescence
683 (LIF), electrochemical (ED) and other detections in food microfluidics **(B)**. All the data
684 were obtained from Web of Science (Thompson Reuters) from 1990 to date
685 considering articles and reviews.

686 **Figure 2.** Strengths and weakness of *Food Microfluidics*. General strengths and
687 weakness are point out in black, the specific ones related to *Food Microfluidics* in red.

688 **Figure 3.** Conceptual and realistic solutions departing from key-technology reservoirs
689 flowing towards *Food Microfluidics*

690 **Figure 4.** Carbon nanotubes press-transferred on PMMA substrates as exclusive
691 transducers for electrochemical microfluidic sensing. Reprinted with permission of
692 reference [26]

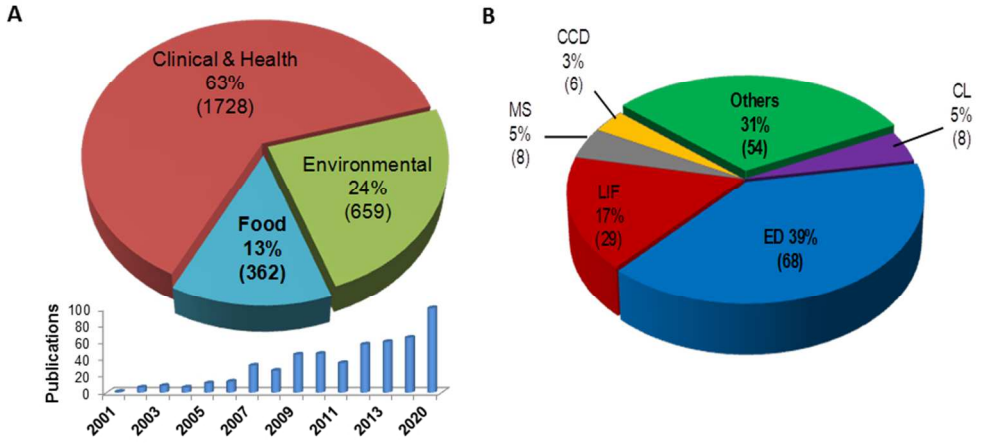
693 **Figure 5.** *Making baby food safer.* Microchip layout and immunoassay principle. (IRC:
694 immunological reaction chamber; ERC: enzymatic reaction chamber). Reprinted with
695 permission of reference [29]

696 **Figure 6**. Microfluidic chips for *Food Microfluidics*.

697 **Figure 7.** Fast flight of microfluidic chip over cereal lands seeking hidden zearalenone
698 mycotoxin when calibration (in blue) and analysis (in red) is sequentially measured
699 using both reservoirs Reprinted with permission of reference [32].

700 **Figure 8**. Scheme of the microfluidic chip used in connection with the screening and
701 confirmation strategy proposed (RB, running buffer; SR, sample reservoir; SW, sample
702 waste; ED, electrochemical detector). EVA (peak 1), VAN (peak 2), PHB (peak 3),
703 VANA (peak 4), and PHBA (peak 5). Reprinted with permission of reference [33].

704



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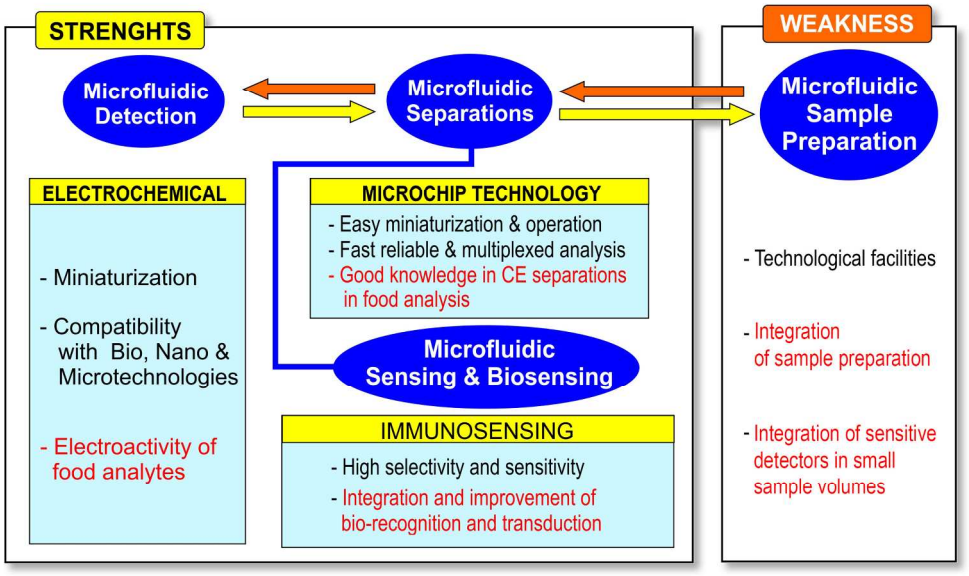

Figure 2. Strengths and weakness of Food Microfluidics. General strengths and weakness are point out in black, the specific ones related to Food Microfluidics in red.
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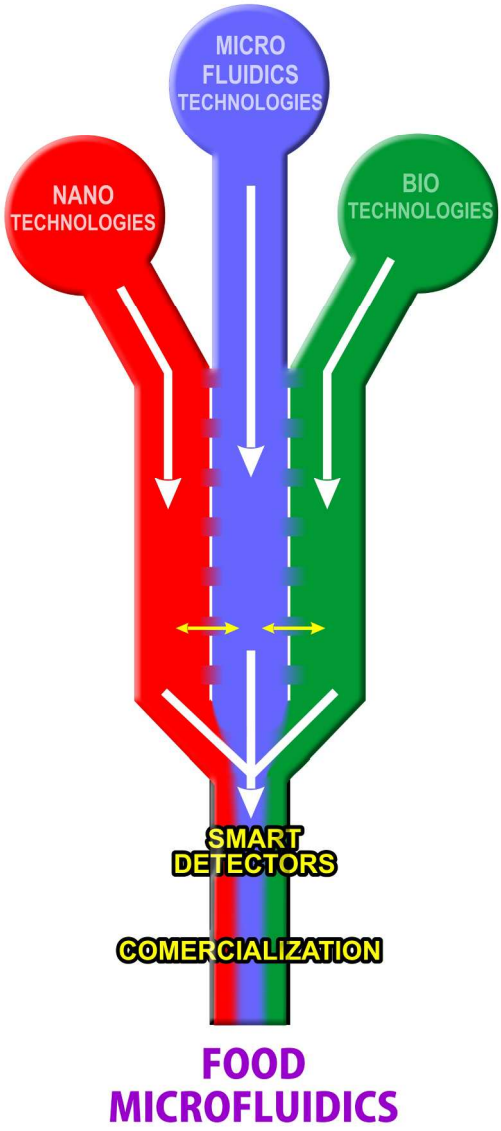

Figure 3. Conceptual and realistic solutions departing from key-technology reservoirs flowing towards Food Microfluidics
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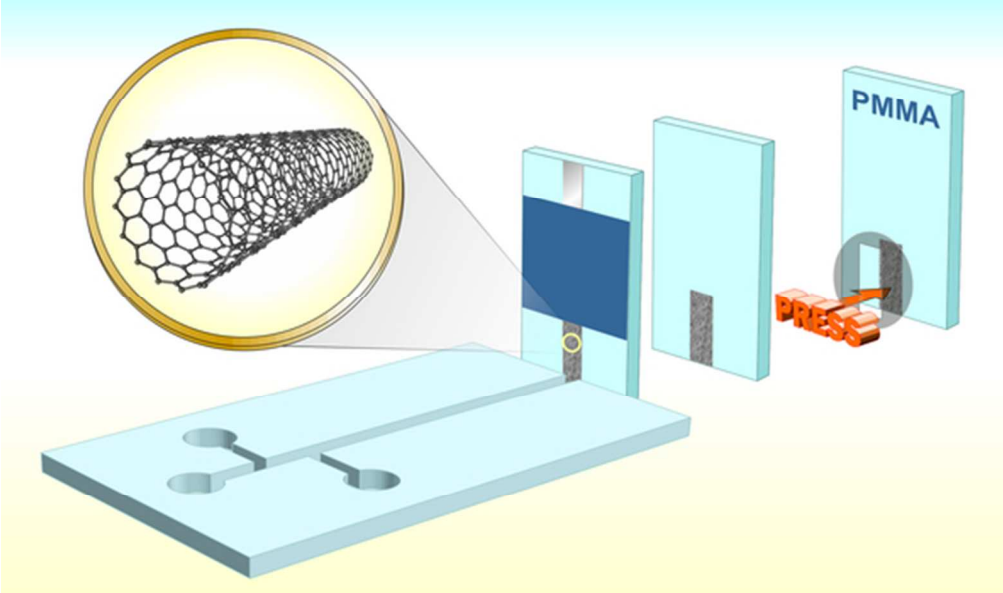

Figure 4. Carbon nanotubes press-transferred on PMMA substrates as exclusive transducers for electrochemical microfluidic sensing. Reprinted with permission of reference [26]
50x29mm (300 x 300 DPI)

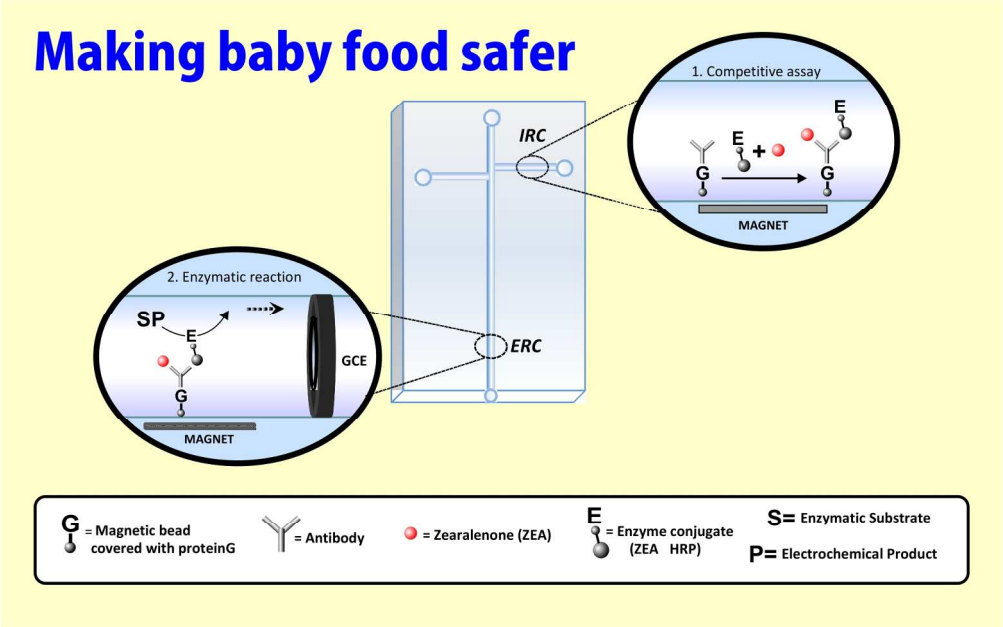

Figure 5. Making baby food safer. Microchip layout and immunoassay principle. (IRC: immunological reaction chamber; ERC: enzymatic reaction chamber). Reprinted with permission of reference [29]
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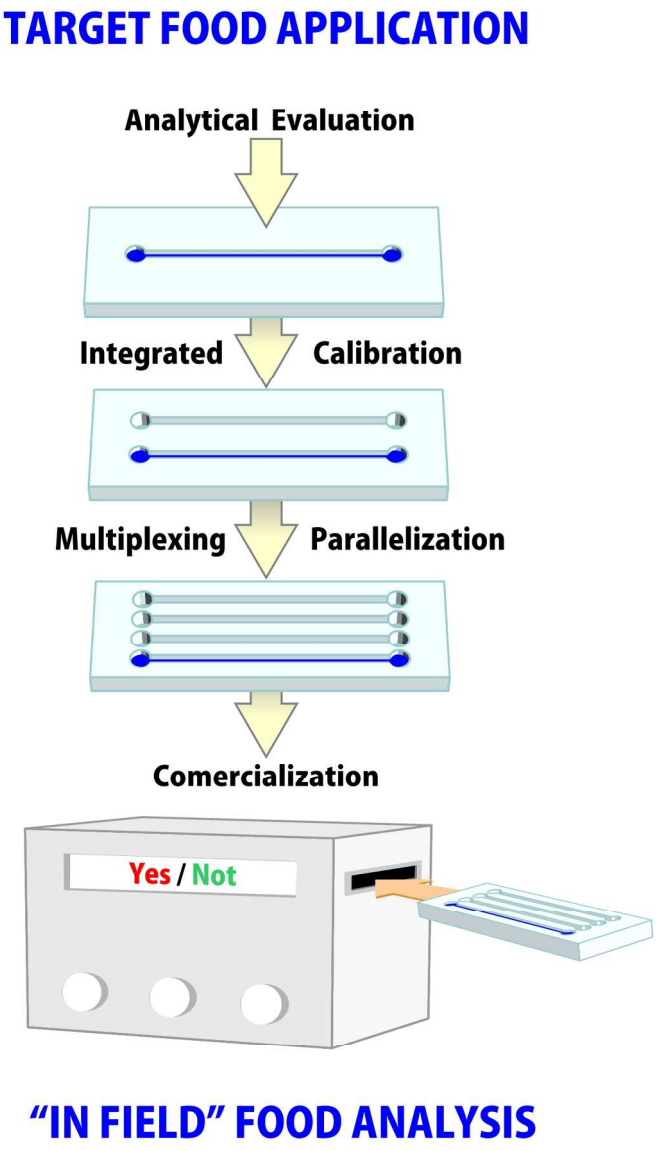

Figure 6. Microfluidic chips for Food Microfluidics.
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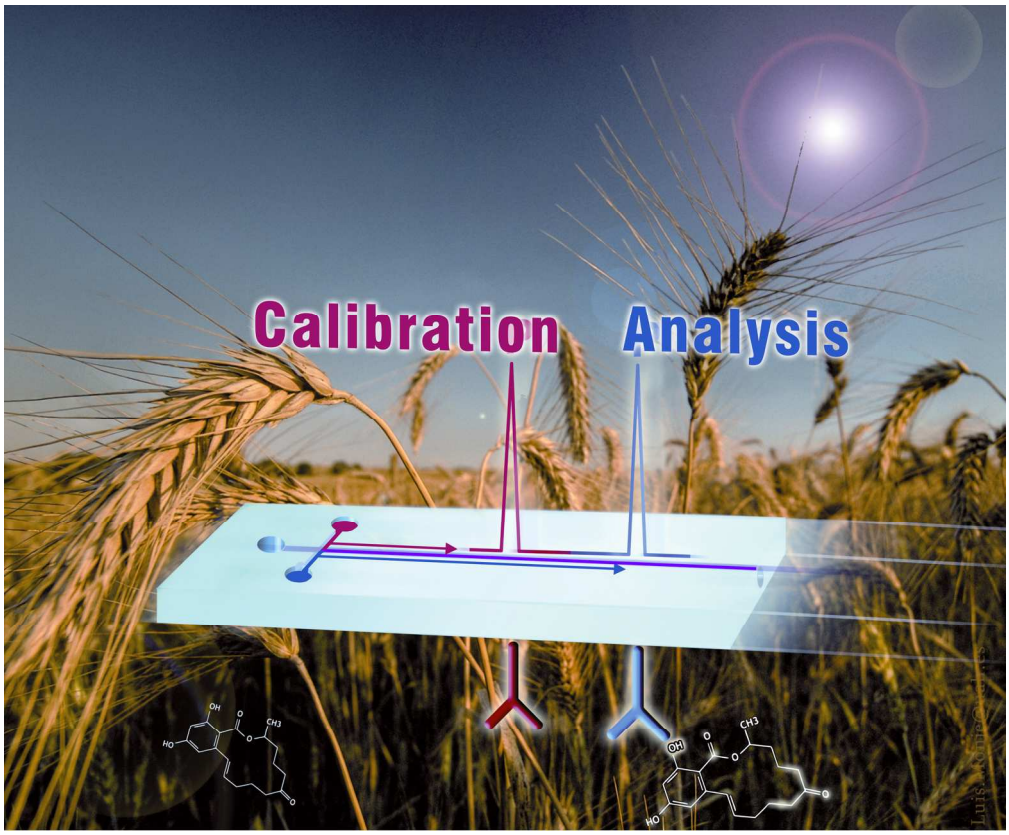
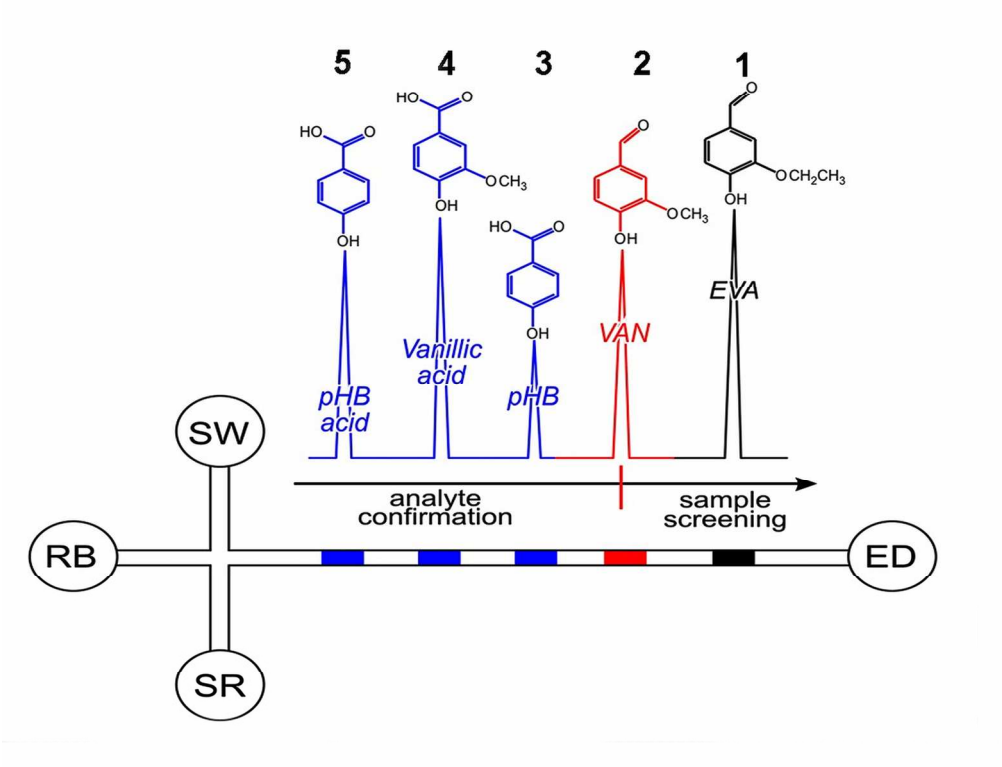


Figure 7. Fast flight of microfluidic chip over cereal lands seeking hidden zearalenone mycotoxin when calibration (in blue) and analysis (in red) is sequentially measured using both reservoirs Reprinted with permission of reference [32].
175x144mm (300 x 300 DPI)



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74x56mm (600 x 600 DPI)