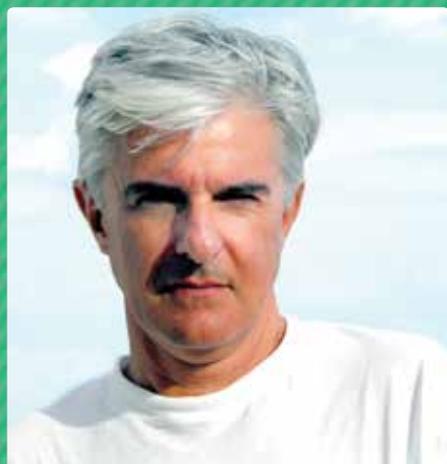


Supplement safety

Professor Massimo Maffei outlines how prevailing misconceptions regarding dietary supplements can be harmful for consumers and how companies like Biosfered Ltd are helping to facilitate informed decisions



Can you outline your background and what led to your research on dietary supplements?

I am a plant physiologist and have studied secondary plant products since 1987 when I undertook postdoctoral research at Washington State University's Institute of Biological Chemistry in the US. My fields of expertise are terpenoids and phenolics, which I research using various different methodologies, from analytical chemistry/biochemistry to molecular biology.

I entered the world of dietary supplements in 1998, when – as an expert in plant bioactive compounds – I was invited by the Office of Dietary Supplements (ODS) of the National Institutes of Health (NIH) to take part in a survey on the main US institutions involved in functional foods studies.

How would you define a dietary supplement? Why have these products become so widely used in recent years?

There are many definitions, but I think that the ODS' definition is the most complete: any substance that is consumed in addition to the regular diet, excluding food items that are intended to be a sole source of nutrition, meal replacements or conventional foods. Dietary supplements are

intended to supplement the common diet and are usually concentrated sources of nutrients. These commonly include vitamins and minerals, or other substances with a nutritional or physiological effect, in particular but not limited to amino acids, essential fatty acids, fibre and extracts of plant origin. Perceptions that dietary supplements are natural and safe and may prevent disease, replace prescription medicines or make up for a poor diet have played an important role in their increased use.

Why are dietary supplements less safe to consume than is widely believed?

The majority of dietary supplements on the market pass the regulatory checks of country's authorities and are therefore to be considered safe. Consumers should always check the products they buy for the authorisation that puts them on the market. In order to be safe and physiologically useful, dietary supplements must be used in a conscious and informed way, considering their function and their properties, without coming into conflict with the preservation of eating habits and appropriate behaviour as part of a healthy way of life. Since their use is generally without medical supervision, it is essential to consider that potential problems due to herb-drug interactions – pharmacokinetic and pharmacodynamic – may arise.

You have highlighted some of the threats posed by misuse of these products – could you elaborate on these dangers?

In general, fewer and less rigorous studies are available for dietary supplements than for prescription drugs, particularly with respect to randomised controlled clinical trials. Future herb-drug interaction studies should be carried out to determine whether herbal supplements can potentially affect

the pharmacokinetics and the efficacy or safety of chemotherapeutic agents.

How does your work seek to overcome some of the most common issues faced by this industry?

We recently created an academic spin-off (Biosfered Ltd) that aims to join the scientific expertise of our group and the capability to isolate and characterise bioactive compounds with industrial processes, through patented techniques and technologies based on green chemistry that do not involve the use of toxic solvents. The products are chemically characterised and titrated via advanced analytical and spectrometric techniques. This strategy aims to stimulate other SMEs embarking on the journey towards the chemical characterisation and quantification of bioactive compounds.

Your research requires you to work alongside industry. Are there ever any conflicting interests?

The role of research centres and universities is to provide the basic and applied science needed to develop methods and strategies for correct technology transfer to industry. The creation of academic spin-offs makes this transfer easier and faster, bringing together two worlds that often revolve around the same issue: improving quality of life. SMEs are often too small to afford the analytical technology that may boost their products, so the support of research centres and universities may represent the turning point to increase their visibility and profits.

Confusing data

Concerns over the quality of dietary supplements have called into question the regulations surrounding their manufacture. Now, research at the **University of Turin** is demonstrating how forging links between universities and SMEs can vastly improve consumer safety

MARKETED AS 'NATURAL' products, dietary supplements are widely perceived as safe agents with nutritional benefits, able to help prevent disease and even serve as replacements for prescription medicine. More and more, people are using dietary supplements as part of their daily routine. They are even becoming popular among patients suffering serious diseases, such as cancer. It is estimated that one person in five uses them in addition to their prescribed medicine, but natural does not always mean safe.

Defined as any orally administered non-food, non-drug product containing at least one dietary ingredient, such as vitamins or minerals, the widespread use of dietary supplements is unencumbered by medical supervision and sometimes rife with misleading labelling of the products themselves. Concerns over excessive intake of vitamins and minerals has led the European Food Safety Authority (EFSA) to set up a framework to ensure responsible labelling of a product's contents so that consumers can make more informed decisions about their purchases. However, a significant challenge exists in completely characterising the bioactive compounds in raw plant materials with the tools commonly used by manufacturers. Addressing this technological gap, research at the University of Turin in Italy aims to help SMEs in the dietary supplement market by ushering in a new generation of analytical methods for standardisation and the accurate quantification of plant bioactive compounds.

ADVANCING THE INDUSTRY

At the heart of this marriage between industry and basic research is Massimo Maffei, Professor of Plant Physiology at the University's Department of Life Sciences and Systems Biology since 2000. In addition to coordinating the PhD School of Pharmaceutical and Biomolecular Sciences and serving as Editor-in-Chief of the open-

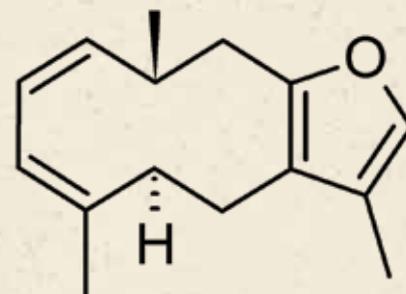
access *Journal of Plant Interactions*, Maffei co-founded the academic spin-off Biosfered Ltd in 2013, in which he acts as the company's Director of Research and Development. Here, Maffei's efforts to develop advanced characterisation and mining tools leave the world of basic research to meet industry, as they are applied to produce bioactive molecules with high commercial value.

Although there are strict regulations for marketing dietary supplements in the EU and the US, too many SMEs lack the funds to acquire the tools needed for more complete analysis of plant raw materials. The two most commonly used methods are hydrogen-1 nuclear magnetic resonance (^1H NMR) spectroscopy and mass spectrometry (MS). With a greater threshold for detecting metabolites, MS is a more sensitive method for characterising bioactive ingredients than ^1H NMR spectroscopy, but surveys show that metabolomic research tends to favour the more insensitive approach of NMR spectroscopy. Without the aid of more advanced analytical techniques for quantification and standardisation, enterprises involved in the business of dietary supplements are prone to miss out the finer details of plant extracts; it only takes one of a variety of factors to induce changes in the composition and contents of their active constituents. Between temperature, climate, humidity, season, soil conditions and various other circumstances, there can be a great deal of variance amongst specimens that are not accounted for by the simple use of spectrophotometers and low-resolution separation techniques. Hence, by joining forces with labs like Maffei's, manufacturers without the means to do so on their own stand a far greater chance of meeting required quality standards.

CUTTING-EDGE CHARACTERISATION

Helping to ensure the validity of manufacturers' claims and improve consumer safety, Maffei's

lab uses state-of-the-art methods for the identification and separation of bioactive compounds from plant extracts. MS and NMR approaches are still vital tools in the research arsenal, but it is the compound of interest that dictates the methods employed. Among these cutting-edge processes are high-performance liquid chromatography (HPLC),



Furanouedesma-1,3-diene: a typical myrrh bioactive furanodiene.



It is estimated that one person in five uses dietary supplements in addition to their prescribed medicine, but natural does not always mean safe

capillary electrophoresis (CE), both gas and liquid chromatography (GC, LC) and diode array detectors (DAD). In combination, these methods can become powerful instruments for generating chromatogram and spectrum 'two-way data', thereby revealing a great deal more about the identities of known and unknown chemical compounds.

Exploiting DAD and MS as identification methods and the separation facility of chromatography, hyphenated combinations of HPLC-DAD-MS instruments are a vital approach employed by Maffei for the analysis of non-volatile compounds. Using GC to initially separate the volatile organic compounds, quantitative determination is then enabled via MS or flame ionisation detection (FID). In a tandem MS (MS/MS) approach that also utilises electrospray ionisation (ESI), Maffei can then analyse the non-volatile compounds using HPLC-DAD-ESI-MS/MS.

However, there is always room for improvement; recently, plans have been made to purchase two instruments: one for ultrahigh performance liquid chromatography-quadrupole time of flight (UHPLC-qTOF) and another for comprehensive two-dimensional gas chromatography-quadrupole time of flight (GCXGX-qTOF). Due to the sensitivity, high mass accuracy and rapidity of data acquisition they afford, time of flight machines will allow for even greater characterisation of both known and unknown bioactive compounds in plant extracts.

CONTENT CONFUSION

Metabolomics and chemometrics are certainly providing the best analyses of raw

plant materials available, but without more stringent labelling rules consumers are often unaware of the realities of the information they are presented with. Some companies label the contents of bioactive compounds in their dietary supplement products as a percentage value while others express the contents in mg per serving or mg per gram of the final product. It is not always clear, however, whether the percentage refers to the chemical analysis or the final weight of the product and is misleading for both consumers and producers.

To illustrate this, Maffei takes the preparation of *Commiphora myrrha* (myrrh) as an example. Myrrh's biological activity is based on its content of furanodienes; expressed as a percentage, the consumer naturally assumes that it refers to the percentage of furanodienes for every kg of myrrh, which it does not. In actuality, the percentage indicates the total amount of volatile compounds in the extract analysed by GC, but there are plenty of other compounds that are not found in the volatile fraction. In order to reflect the true concentration, Maffei believes that producers could use an internal standard in the analytical process with which to quantify the compound and express it in mg/g or kg of the end product.

TRANSPARENCY

Presenting consumers with clearly labelled information is a critical step to give a rationale for the use of dietary supplements. Advanced methods of separation, identification and quantification, obtained with the support of universities and academic spin-offs like Biosfered Ltd, will enable producers in many countries to acquire the highest quality raw-materials to make products that meet international safety standards such as those followed in the EU and US. Some current regulations are looking ever more outdated, as it is increasingly clear that simple spectrophotometer and low-resolution separation methods cannot sufficiently ensure high-quality produce or consumer safety.

Maffei is confident that with the increased use of advanced analytical instruments, regulatory bodies will have to change their protocols in line with growing global awareness on the risks and benefits of dietary supplements.



Myrrh resin-gum: a raw material full of bioactive compounds.

INTELLIGENCE

QUANTIFYING DIETARY SUPPLEMENTS OF PLANT ORIGIN

OBJECTIVES

The overall project goals are to characterise plant metabolomics and transcriptomics of bioactive compounds by means of chemical and genetic analyses under different stress conditions. Specifically, the aim is to provide SMEs with sound and certified technologies and methodologies for the quantitative determination of bioactive compounds in raw materials used for the preparation of dietary supplements.

KEY COLLABORATORS

Professor Klaus Palme, University of Freiburg, Germany

Professor Junji Takabayashi, University of Kyoto, Japan

Professor Gen I Arimura, Tokyo University of Science, Japan

Professor Axel Mithöfer, Max Planck Institute for Chemical Ecology, Germany

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CONTACT

Professor Massimo E Maffei
Professor of Plant Physiology

Department of Life Sciences and Systems Biology
University of Turin
Via Quarellino 15/A
10135 Turin, Italy

T +39 011 670 5967
E massimo.maffei@unito.it

www.biosfered.com

www.plantphysiology.unito.it

MASSIMO E MAFFEI has been Professor of Plant Physiology at the University of Turin, Italy, since 2000, as well as R&D Director of the academic spin-off Biosfered Ltd. He has published around 150 papers in phytochemistry, plant biology, plant physiology and plant interactions with the surrounding biotic and abiotic environment. He has been Head of the Department of Plant Biology, Deputy Dean of Faculty of Sciences and Coordinator of the Ministerial Centre of Excellence CEBIOVEM. He is Coordinator of the Doctorate School of Pharmaceutical and Biomolecular Sciences of the University of Turin and Editor-in-Chief of the open access *Journal of Plant Interactions*.

