AF/04

FOODINTER - Results

Food interactions : effects on health, consumer perception and impact on agro-food industries

DURATION OF THE PROJECT
01/01/2007 – 31/04/2011

BUDGET
720.706 €

KEYWORDS
Food supplement, plant, *Ginkgo biloba*, St John's wort, Maca, Black radish, Garlic, Soy isoflavones, interaction, consumer, environmental contaminant, heavy metal, mycotoxin, PAH, toxin, dioxin, hormone, natural, drug, cytochrome P450, CYP1A1, stress, extract, active ingredient, labelling, notification

CONTEXT

Food supplements (FS) are subject to an ever growing interest, as they are now consumed by an increasing number of people. According to the results of a consumption survey conducted in France by the CCAF (2004), (N=1361), 11% of the grown-up respondents were FS consumers and 59% are consumers of « extended health food/products » (Gaigner, 2005). Botanical materials represent a large segment of this class of products (e.g. soy isoflavones, yam or hop extracts). The vast majority of plant based food supplements sold legally in the EU today is harmless under recommended conditions of use. However, plant extracts as such may not be harmless.

Furthermore severe cases of drug-plant extracts interactions have been well documented on patients (Abad, 2010, Chavez et al., 2005, Colalto, 2010, Izzo & Ernst, 2009), and it is likely that many more cases are not declared because not considered as originating from these interactions. Because of their vegetal origin and, in some cases, the non application of legal manufacturing guidelines (especially for materials prepared in third countries), the raw material used in preparing plant-based food supplements might also carry toxic environmental contaminants such as heavy metals or polycyclic aromatic hydrocarbons. It is important that plant-based FS comply with the legislation. Numerous legal requirements are in place in the EU to ascertain that food of vegetal origin does not contain potentially harmful amounts of residues or contaminants.

OBJECTIVES

The Foodinter project aims to study selected food supplements (FS) sold on the Belgian market in terms of chemical contamination and the activity of their active ingredients. Six categories of plant derived products were chosen : gingko-biloba, St John's wort, soy isoflavones, maca, black radish and garlic.

The objective of this project is thus to contribute to the risk assessment of chemicals, natural compounds and environmental contaminants, present in FS which could interact between each other or with micro- or macronutrients of normal human diet. Interaction studies have been performed using existing *in vitro* models (based on various cultured cell types, prokaryotes and eukaryotes) with mixtures of active substances at concentrations that are very close to the real situation in human nutrition.

Furthermore, this project aims to promote the communication between scientists and stakeholders (authorities, producers and consumers). In the field of food consumption, this objective is important because food safety depends not only on production and control, but also on consumption practices and good information must therefore be promoted. The objective is not only an educational planning, but also to promote a dialog between science and society in order to better identify the societal preoccupations and needs that research has to satisfy.

CONCLUSIONS

Our project was divided in 3 parts :

1. Selection and chemical analysis of samples of food supplements found on the Belgian market,
2. *In vitro* analysis of pure compounds identified as active ingredients of the selected food supplements, as well as food supplements extracts,
3. Study of the consumer perception of food supplements using the techniques of surveys, at the beginning of the project, and focus groups, at the beginning and the end of the project

Selection and chemical analysis of samples of food supplements found on the Belgian market

We have selected for this project six different FS all made from one specific plant material:

- Ginkgo biloba: improve blood circulation and cerebral oxygenation
- St-John's wort (*Hypericum perforatum*): for mild depression
- Soy isoflavones (from *Glycine max*): reduce menopause effects
- Maca (*Lepidium meyenii*): increase libido and limit sexual disorders
- Black radish (*Raphanus sativus*): for bile secretion and intestine activity
- Garlic (*Allium sativum*): decrease arterial tension

In order to have an idea of what the consumer can find on the Belgian market, 61 samples were collected, purchased from 37 companies via internet (36 samples), pharmacies (18 samples) and specialized shops (7 samples). Twenty five FS were notified in Belgium whilst 36 were not notified (and generally available via the internet). This material was used to perform the analyses of chemical contaminants and active ingredients.

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Analysis of chemical contaminants

Analysis of mineral éléments : Seventeen trace elements (As, Ba, Bi, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Sr, Ti, Tl, Zn) were quantified by inductively coupled plasma with mass spectrometer (ICP-MS). Mercury (Hg) was quantified by Advanced Mercury Analyzer (AMA).

There were 10 non compliant (NC) samples with respect to the Belgian legislation in force in 2007, at the time the samples were bought, for toxic element in FS (7 NC for Pb and 4 NC for Cd; one sample exceeded the maximal limit for both elements). Since 2008, the Belgian legislation has been replaced by a European Regulation, with higher maximal limits for Pb and Cd, and only 4 samples, ut of thèse 10, would remain NC for their Pb content.
Analysis of mycotoxins : The target mycotoxins included nivalenol (NIV), deoxynivalenol (DON), neosolaniol (NEO), fusarenon-X (F-X), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), diacetoxyscirpenol (DAS), HT-2 toxin (HT-2), T-2 toxin (T-2), aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), ochratoxin A (OTA), altenuen (ALT), alternariol (AOH), alternariol methylether (AME), fumonisin B1 (FB1), fumonisin B2 (FB2), fumonisin B3 (FB3), zearalenon (ZEA), beauvericin (BEAU), sterigmatocystin (STERIG). They were analyzed using gradient reversed-phase liquid chromatography (RP-LC) with electrospray ionization tandem mass spectrometry (ESI-MS/MS).

The toxins FB1, FB2, FB3 and OTA were detected in some samples. In 2 samples (one of Gingko Biloba and one of Maca), OTA was found at a level above 2 µg/kg, the EC maximal limit for wine and grape juice, according to Regulation 1881/2006/EC (this limit was taken as a reference, as there are no maximal limits for food supplements). The levels of FB1, FB2 and FB3 were largely below 800 µg/kg (EC norm for the sum of FB1 and FB2 in breakfast cereals, Regulation 1881/2006/EC) in all samples.

Analysis of PAHs : High performance liquid chromatography coupled to an ultraviolet, diode array or fluorescence detector (HPLC/UV-FLD) has been used to detect the 15(+1) EU priority PAHs in the sixty food supplements selected in this project. The results have shown that St-John's wort and *Ginkgo biloba* extracts presented the most frequent contaminations and the highest average values for PAHs concentrations. The most contaminated samples with the sum of the 16 PAHs were generally detected in St-John's wort and ginkgo products, except one sample of soy isoflavones. However, in most cases, the PAH intake from FS is less than 5% the PAH intake from normal food.

Analysis of organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and of polybrominated diphenyl ethers (PBDEs), and dioxins in oily FS : Low amounts of p,p'-DDE or p,p'-DDD (< 10 µg/Kg) were detected in 3 garlic samples, but far below the legal limit of 50 µg/Kg for the sum of DDT. Dioxins, PCBs and PBDEs were below the limit of quantification of the assay.

Identification and analysis of active ingredients
Relevant active ingredients were identified from the literature in the 6 categories of food supplements.
These active ingredients are listed below.

St John's wort	Ginkgo biloba	Soy isoflavones	Black Radish	Garlic	Maca
Hypericin	Ginkgolide A	Genistein	L-sulforaphane	Garlic oil	lepidilin A
Hyperforin	Ginkgolide B	Daidzein	DL-sulforaphane	S-allyl cysteine	Lepidlin B
	Ginkgolide C	Glycetein	Glucoraphanin	Allicin	Macaridin
	Ginkgolide J				MTCA
	bilobalide				
	Isorhamnetin				
	kaempferol				
	Quercetin				

For some of them, these active ingredients have been analyzed using chemical methods (HPLC or LC-MS) in order to determine the real content of the chosen FS in these active ingredients.
Analysis of six maca products (3 notified, 3 not notified) by nano-LC-MS revealed one product containing no lepidilin A and no lepidilin B. From these data, it could be concluded that this sample does not resemble a maca-extract. Nevertheless, macaridin was detected in all maca supplements, with no correlation with the presence of lepidilins.

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The glucosinolate (GL) profile in black radish (*Raphanus sativus)* based dietary products was investigated. An analytical strategy combining the use of LC-PDA, LC-ESI-MS/MS and LC-APCI-MS/MS systems was applied. The LC-ESI-MS/MS system was used to detect and identify the naturally occurring intact GLs. The identified intact GLs were then desulfated and quantified on an LC-PDA system as desulfo-GLs. Prior to quantification, the desulfo-GLs were identified using an APCI-MS/MS system. In total, six glucosinolates were identified and determined (quantified) in the six analyzed products. The quantitative data revealed a great diversity in the individual GL which can be attributed to differences between species and subspecies of the different black radishes from which the products were derived. Different growing conditions could also have contributed to the differences in the glucosinolate content in the different samples.
According to the literature, glucoraphenin was found the most abundant glucosinolate in all samples.
The hypericin content specified on the packaging of thirteen different food supplements was registered. In five cases (not notified products), the announced content of hypericin leads to an intake higher than the legal limit of 700 µg/day in the “plant” Royal Decree of 1997 (MB, 1997). We also observed the lack of hypericin content information on two notified food supplements.

The analysis of active ingredients in the selected products shows that the content of the product is not always mentioned on the label. This is mandatory, according to the European Directive 2002/46/CE, transposed in Belgian legislation in the “plant” Royal Decree of 1997. But, as this “plant” decree is not yet complete, because the active ingredients are not identified for each plant food supplement, this obligation is not applicable.
This shows the need for more research to identify active ingredients in plant FS as well as a gap to fill in the European and Belgian legislation.

In vitro analysis of pure compounds identified as active ingredients of the selected food supplements, as well as food supplements extracts

For the *in vitro* study, we focused on 3 categories of products, on the basis of the frequency of their consumption : soy isoflavones, St John Wort and *Ginkgo biloba*. For these 3 categories of products, we have tested their active ingredients separately with our panel of *in vitro* tests, as pure standards. The complete study (*in vitro* testing of active ingredients separately, mixture of active ingredients and plant extract) has been performed only on *Ginkgo biloba*, using a reference material (from the National Institute of Standardization, NIST), with certified concentration of active ingredients of *Ginkgo biloba*.
In order to work with plausible intestinal concentrations, we have estimated daily intake (EDI) of each selected compound, using FS content values either from literature or from packaging. For certain compounds, such as active ingredients from soy isoflavones, the EDI from the diet must be added to the EDI from food supplement consumption. This methodology provided us with an idea of plausible intestinal concentrations to use as reference concentrations in our *in vitro* intestinal barrier model.
In all *in vitro* models, ranges of concentrations were tested, including the intestinal plausible concentration, as a reference.

The *in vitro* models used where :

- A model to study the general toxicity of the active ingredients in bacteria (*E.coli*) and eukaryotic cell lines (hepG2 and Caco2);
- A model to study the possible toxic mode of action of active ingredients using bacterial reporter gene assays allowing to investigate four mode of action : oxidative damage, DNA damage, general cell lesions and membrane damage;
- A model to study the possible endocrine disrupting and dioxin-like activity of active ingredients using eukaryotic reporter gene assays (where the interactions with respectively, human steroid receptors and rat and human aryl hydrocarbon receptors, were studied);
- A model to study the possible effects of active ingredients on human P450 (CYP) 1A1 activity in human colon adenocarcinoma cells (Caco2 cells).

The main results obtained with active ingredients at concentrations equal or below their calculated intestinal plausible level are indicated here below :

In **general toxicity assays**, some cytotoxicity was observed with hyperforin, bilobalide, isorhamnetin, kaempferol, genistein, daidzein and glycitein.
In **mode of actions (MOA) assays**, hypericin and hyperforin showed no effects, while an effect on DNA damage MOA was recorded for Gingkolide A, kaempferol and genistein, on cellular stress MOA for kampferol and on oxidative damage MOA for kaempferol, genistein and daidzein.

In the **Caco2 model to study the CYP1A1 activity**, hypericin was able to slightly induce the CYP1A1 activity as well as to inhibit the CYP1A1 induction in presence of BaP, while hyperforin was only able to slightly induce CYP1A1 activity.

The terpens from *Ginkgo biloba* (Gingkolides and bilobalide) as well as one flavonol (isorhamnetin) had no effect on the CYP1A1 activity, while the two other flavonols tested (kaempferol and quercetin) were able to induce as well as to inhibit the CYP1A1 activity. The whole *Ginkgo biloba* extract (NIST reference material) displayed the same kind of effect, but with a lower intensity.

Soy isoflavones (genistein, daidzein and glycitein) were able to inhibit CYP1A1, but not to activate it.

In the **study of the interaction with the aryl hydrocarbon receptor (AhR),** specific species effects were recorded. Both hypericin and hyperforin displayed the same effects : while they were unable to activate both the rat and human AhR, they potentiate the inductive effect of the reference ligand TCDD (tetrachlordibenzodioxin) in rat hepatoma cells (synergistic effect), but they inhibit this inductive effect in human hepatoma cells.

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For terpens active ingredients of *Ginkgo biloba*, only Gingkolide J displayed an effect (activation of human AhR), and for flavonols, only quercetin was able to activate the AhR (in human but not rat hepatoma cells), while the three flavonolos (isorhamnetin, kaempferol and quercetin) inhibited the AhR (in both rat and human cells for isorhamnetin and quercetin and only in human cells for kaempferol).

The NIST *Ginkgo biloba* reference extract induced the AhR in both rat and human cells, acted in synergy with TCDD to induce AhR in rat cells (no inhibition effect) and inhibit the TCDD induction of AhR in human cells. This last result is concordant with the observation made in Caco2 cells, where the NIST *Ginkgo biloba* extract both slightly induced the CYP1A1 (which is expressed in response of the activation of the Ah receptor, which is a transcription factor) and inhibited the CYP1A1 induction by BaP, another reference ligand for the Ah receptor.
For soy isoflavones (genistein, daidzein and glycitein), the results obtained when studying their effect on the Ah receptor are less concordant with the observations made on the CYP1A1 activity: the three isoflavones were able to induce the rat AhR but not the human, while genistein and daidzein acted synergically with TCDD to induce AhR in rat cells and only genistein inhibited the TCDD induction of AhR in human cells.

When studying the **possible endocrine disrupting activity of selected active ingredients**, hypericin and hyperforin were not able to activate steroid receptors (human estrogen, androgen, glucocorticoid and progesterone receptors), while hypericin inhibited the induction of the glucocorticoid receptor and both hypercin and hyperforin were able to inhibit the induction of the human estrogen receptor, showing a possible anti-estrogenic activity.

Terpens showed no activity on steroid receptors, while the 3 flavonols were able to induce the human estrogen receptor (showing an estrogenic activity) but also to inhibit the induction of the progesterone receptor (anti-progestagen activity).

The soy isoflavones, known to display an estrogenic activity, showed an expected activation effect on the estrogen receptor (showing that our model works), but also an inhibitory activity of the less studied progesterone and glucocorticoid receptors, showing a possible effect of these substance on other steroidal pathways than the estrogenic one.

To summary the first two parts of the project, the results show that potential risks, for public health, are linked to 3 major areas: interactions with drugs that may modulate their efficiency (shown from the literature study), environmental contaminants, such as heavy metals and polycyclic aromatic hydrocarbons (shown from the analysis of 61 samples bought on the Belgian market in 2007), and biological effects of certain active compounds that might for example act as endocrine disruptors for specific target groups or modulate the activity of the metabolizing enzyme CYP1A1.
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Study of the consumer perception of food supplements using the techniques of surveys, at the beginning of the project, and focus groups, at the beginning and the end of the project.

From the consumers' encounter (third part of the project), we draw the following conclusions : the first is that **consumers do not exactly know what can be categorised as FS** and what can not. This raises the problem of a poor knowledge in the public of the exact definition and status of those products, even if paradoxically the categorization and definition of all health products seems heavily framed through regulation.

The second is that **FS (or products sold as FS) consumers are not a homogeneous group**; they have very different profiles and may share very few characteristics from one to another. Food supplements consumption is widespread among the population, but the monthly expense on those products generally ranges between 20-50€. The four main profiles we identified are: performance profile ; well-being profile ; deficiency profile ; prevention profile.

The third is that **consumers have generally low risk awareness** (even lower for interaction risks), lack a lot of information and of critical distance on those products, but want to be better informed. They also often make self-research on products, and can sometimes become hard to challenge (even by experts) as their main source of faith is themselves, the information they collected, their own experiences and feelings, or coming from trusted relatives. However, they can't be dismissed as "irrational" as they root their choices and judgements in personal history, values, “documented fears” (such as generalised low food quality), etc.

The fourth is **that the status to give to consumers in face of risk management should be clarified and reflected on** ; indeed, consumers could play important roles regarding risk governance and should not simply be envisaged as passive receptors of information.

The fifth is that **strategies of risk management that only rely on control show their limits** in face of the new nature of risks, mainly complexity/systemic nature and uncertainty. Therefore, new strategies and new models of risk governance should be designed and experimented

Several actions could be envisaged in order to decrease possible negative health impacts. For example, the implementation of the HACCP-guide for food supplements that has been developed by the Belgian food supplement sector and has been approved by the Belgian authorities (guide G-011 approved on 9/08/2007). This guide is a guideline for producers on how to implement a quality control system covering all the steps: from growing conditions through transport to preparation and conservation, using the HACCP approach (for Hazard Analysis Critical Control Point).

This is already mandatory in Belgium, following the “Autocontrol” Royal Decree of November 2003 (Moniteur Belge, 2003), which is an implementation of the European Food Law (Regulation (EC) n° 178/2002). In this HACCP plan, **producers should reinforce their attention on chemical hazards**, such as heavy metals (included at the moment in the European Legislation, but not when the project started), as well as polycyclic hydrocarbons (not yet included in the European Legislation). Targeted chemical controls might be envisaged depending on the plant species.

From the consumers' enquiry and the discussions in focus groups, we can make the following **recommendations** : the first is that the **communication strategy should take into account the diversity in consumer profiles and consumption patterns**. Therefore, a multifaceted communication strategy would be more suited than a too generalist and simplistic one.

The second is that an **Internet platform (assembling competent authorities, representative of industry, scientists and consumer's organizations) on risks associated with FS (and products sold as FS) consumption** would be a potentially very useful and powerful tool for consumers' information and empowerment, but also for risk governance, knowledge sharing with health professionals and mediators, and associated interdisciplinary research.

The third is that **health professionals (practitioners, specialists of nutrition, dieteticians, pharmacists, etc.) should follow more training.** Food supplements, alternative health products and nutrition (including problems of interactions between food and drugs) should be an essential part of their training. If those competences aren't mastered by practitioners (like patients complain about), then shouldn't the consultation of specialists of nutrition or FS be refunded by the healthcare system ?

The fourth is that **producers should give even more (and better) information on products and processes**, but also on theirs associated risks (including potential interactions with personal specificities, medicine and food).

The fifth is that **FS management and associated regulation, procedures or product categories should be, if not reworked, at least clarified for consumers** and allowing them to be more “responsible” actors in the risk management system.

CONTRIBUTION OF THE PROJECT TO A SUSTAINABLE DEVELOPMENT POLICY

This project would like to draw the attention of the authorities and all stakeholders on the risks linked to the free consumption of certain categories of food supplements, especially derived from plants.

All the recommendations described above (to improve : - quality control systems in FS production plants, - the information to the consumers, - the training of health professionals, - the communication between stakeholders) would result in a better management of public health in Belgium.

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