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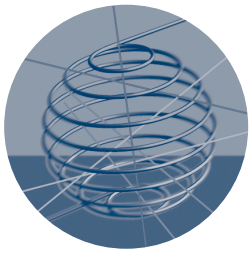
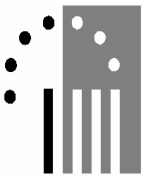


PRENORMATIVE RESEARCH IN THE FOOD SECTOR

SCIENTIFIC SUPPORT PLAN FOR A SUSTAINABLE DEVELOPMENT POLICY SPSD 1

This booklet is realised in the framework of the Scientific Support Plan for a Sustainable Development Policy (SPSD I). The available publications are :

- *"Antarctica"*
- *"Levers for a sustainable development policy"*
- *"Earth observation by satellite" TELSAT 4*
- *"Pre-normative research in the food sector"*
- *"Global change and sustainable development"*
- *"Sustainable management of the North Sea"* (available from spring 2003)
- *"Sustainable mobility"* (available from spring 2003)
- *"Supporting actions"* (available from spring 2003)



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PROGRAMME " PRE- NORMATIVE RESEARCH IN THE FOOD SECTOR "

The scientific support programme for pre- normative research in the food sector and within the sustainable development concept aims to put forward evaluation tools that allow the definition of political orientation in the fields of quality of life (consumer protection) and the optimal use of resources (environmental protection).

Two parts whose objectives are described below compose the programme.

Part 1

Inventory of fixtures of the normalisation of products in the food sector with a sustainable development context in Belgium and abroad.

The selected projects' results should allow:

- the inventory of activities that concern the development of standards and the settings of limits for pollutants or harmful effect levels that should not be exceeded in composition or emissions of the food products; inventory of control tools developed by industry and Belgian public research centres;
- on the basis of this inventory, to recreate the networks and so contribute to the integration of results;
- to identify the fields of the food production system for which a sustainable development normalisation effort should be sustained;
- to run an inventory of European and international initiatives;
- to develop a database accessible for all;
- to examine the actions undertaken at a Belgian and international level in terms of food standards and sustainable development;
- to define the Belgian contribution to the international (more so European) effort.

Part 2

The selected projects' results should allow:

- to fill the gaps in the scientific knowledge and to contribute to the definition and application of standards for the whole of the production network to maintain a high level of required expertise;
- our country to take part actively in the elaboration of standards within international organisations in order to protect the environment.

More so, the pilot projects must firstly allow the implementation of standards and secondly they must promote the elaboration of better measuring and testing methods. These are the bases for the definition and application of norms.

- the definition of evaluation and control criteria's for production linked nuisances aimed at the elaboration of new product standards in a sustainable development context;
- the applicability of life cycle analyses in the context of the elaboration or adaptation of products standards;
- the development of control methods that insure the products ' authenticity;
- the setting up of awareness and stimulation activities aimed at the private sector, consumer associations, research centres and public administrations.

These objectives can be reached in various ways such as, for example, demonstration production projects (small scale) which respect the environment, the elaboration of new control methods; awareness actions aimed at targeted audiences, the setting-up of expertise networks, etc.

Only the syntheses of the projects of the programme are to be found in this document.

Complete final reports are accessible on the web-site (http://www.belspo.be).

NP/DD/01-02-03

STANDARDISATION OF HORMONE AND VETERINARY DRUG RECIDUE ANALYSIS IN ANIMAL PRODUCTS

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1. SUMMARY

A data bank is created which provides a critical inventory of analytical methods available for the analysis of residues of growth promoters (steroidal anabolic hormones, ß-agonists and glucocorticoids) and veterinary drugs (antibiotics and growth inhibitors), which are or will be regulated by the European Regulations, Directives and Decisions. This data bank will be available on a telematic network (e.g. Internet).

The European Union prescribes its Member States to monitor certain substances and residues thereof in live animals and animal products (Directive 96/23/EC) (1). Two groups of substances are included in this Directive: group A: substances having an anabolic effect and unauthorized substances and group B: veterinary drugs and contaminants. Group A involves certain substances having a hormonal or thyrostatic action and ß-agonists in stock farming (Directive 96/22/EC) (2) and veterinary drugs now forbidden (Annex IV of Council Regulation (EEC) No 2377/90) (3). The aim of this project was to establish an inventory, in the framework of the directives and regulations cited above, of analytical methods for residues of specific classes of growth promoters (sex hormones, ß-agonists and glucocorticoids) and veterinary drugs (antibiotics) which after validation could be standardized. This inventory is organized under the form of a data bank, which will be accessible via electronic means (e.g. Internet). The data bank contains information about the following topics:

1. A compilation of analytical methodologies for the screening and confirmation of residues of growth promoters and veterinary drugs

2. A compilation of chemical and physical data of residues found in foodstuffs of animal origin

3. An inventory of the European and Belgian legislation with regard to residues in animal products

4. An estimation of the consumption of animal products in Belgium

5. Qualitative and quantitative data about the monitoring programs by the Belgian Institute for Veterinary Inspection and the Belgian Ministry of Agriculture

6. A compilation of toxicological data as to consumers health

7. A critical evaluation of the analytical methods for the analysis of residues

8. A list of available reference materials and specific reagents (antisera, immunoassay kits, immuno affinity products, radioactive tracers...)

In practice a monitoring program is often divided into a screening phase and a confirmation phase. In the first phase a large-scale screening is put into place allowing the analysis of a large number of samples using a cheap, simple and rapid method. Immunoassays (radio- and enzyme immunoassays) are well suited to this purpose.

Screening methods yield positive or negative results. Negative results are generally accepted as such whereas positive samples must be confirmed in a second phase to distinguish between true positive results and false positive results. Analytical methods based on chromatography, most often coupled to spectrometry (UV, FT-IR, mass spectrometry) are methods of choice for the second phase (confirmatory analysis).

Despite the fact that many methods for both screening and confirmation have been published, very few, if any, have been tested on an interlaboratory scale. Till a few years ago an analytical method was considered suitable only after successful testing in a collaborative study involving a circular analysis of an identical sample in several laboratories.

This view has now changed owing to the high cost of such studies, the long time needed to perform the test, the rapid progress made in the development of analytical methods and the high number of residues for which analytical methods are needed. Within the European Union criteria that must be fulfilled have been fixed (Commission Decisions 93/256/EC (4) and 93/257/EC (5)). These criteria are very helpful to verify the sound basis of a method and provide guidelines for the analyst who develops methods others than reference methods. The approach by these criteria does not mean that interlaboratory testing is no longer needed. Circular analysis is the ultimate test to demonstrate the quality of a method and it is a very efficient tool in the training of laboratories and in the harmonization of the quality of their work.

The analytical methods, which are included in the data bank, were critically evaluated according to the criteria described in Commission Decisions 93/256/EC (4) and 93/257/EC (5). These Commission Decisions prescribe definitions to be met for screening tests and confirmation analyses, analytical methods which may be used and detailed criteria which should be met by the techniques. Aspects such as specificity, limits of detection and determination, accuracy and other quality criteria are discussed. After evaluation the methods were classified into two categories:

* A: high reliability: method is in accordance with the prescribed criteria

* B: limited reliability: method is partially in accordance with prescribed criteria

Analytical methodologies for the screening and confirmation of residues were obtained through a systematic search of the international scientific literature and completed by data obtained from the Proceedings of specialized scientific meetings, especially dedicated to the subject. For the analytical methods included in the data bank, the following information is provided:

* bibliographic references (authors, journal, publication year, address of the first author...)

* sample type (e. g. plasma, urine, kidney, muscle...) used to evaluate the method

* residues which can be detected and/or confirmed by the method

* sample preparation (extraction type, clean-up, fractionation...)

* method conditions

* limit of detection and/or limit of quantitation

* grade (A or B)

Control of residues, particularly growth promoters, in animal products is performed by two governmental departments:

* Department of Public Health: the Institute for Veterinary Inspection is responsible for collecting samples in slaughterhouses and occasionally in farms, the Inspection Service for Foodstuffs is responsible for sampling in butcher shops

* Department of Agriculture in farms, Veterinary Inspection and Inspection of Raw Materials

Analyses are performed by approved laboratories. The hierarchy of laboratories in charge of residue control in the European Union is well defined (Directive 96/23/EC) (1). There are four Community Reference Laboratories (CRL) that are responsible for certain categories of substances. These CRLs are in charge of the control and the training of the National Reference Laboratories (NRL). In Belgium, the Scientific Institute of Public Health - Louis Pasteur is responsible for the analysis of all types of residues in live animals and animal products. Other Belgian laboratories (about 6) are involved in the routine analysis of residues especially growth promoters. They are supervised by the NRL. The results of the monitoring programs conducted by the

Institute for Veterinary Inspection in Belgium for 1994, 1995 and 1996 are presented in the data bank.

Chemical and physical information about the residues found in food products of animal origin are included in the data bank. For all the compounds discussed the following information is provided: therapeutic category, chemical abstracts name and synonyms, chemical abstracts registry number, number of references of methods included in the data bank for the determination of the compound, molecular formula, molecular mass, structure, derivatives and toxicological data. A compilation of physical data (e. g. solubility, optical rotation, molar absorptivity, melting point...) is, when available, included for each compound.

Several European regulations and directives were recently adopted. They prescribe the Member States to control certain substances and their residues in living animals and their products (Directive 96/23/EC) (1). Two groups of substances are concerned by this directive: (A) substances having an anabolic effect and other unauthorized substances and (B) veterinary drugs and contaminants (annex I of the directive). Group A involves certain substances having a hormonal or thyrostatic action and ß? agonists in animal production (Directive 96/22/EC) (2). Council Regulation (EEC) No 2377/90 (3) laying down a community procedure for the determination of maximum residue limits (MRLs) for veterinary drugs in foodstuffs from animal origin is in progress. For the control of the application of these regulations and directives and of the corresponding Belgian legislation a program for the control of residues, concerning mainly substances illegally used as growth promoters and veterinary drugs including unauthorized substances which could be used as veterinary drugs, must be put into place every year and executed by Member States of the EU after approval by the Commission.

A survey of the Belgian consumption of meat (kg/inhabitant/year, expressed as carcass weight) for the last 40 years is presented for different animal species. The data were obtained from the Belgian Ministry of Agriculture.

2. CONCLUSION

A data bank was created containing a critical inventory of analytical methods, which after validation may be considered as candidates for standardization. The methods were classified as "High reliability" or "Limited reliability" according to their conformity with the criteria described in Commission Decisions 96/256/EC (4) and 96/257/EC (5). The data bank also contains toxicological data as to consumers health, an estimation of the Belgian consumption of meat, an inventory of the European and

Belgian legislation with regard to residues in animal products, commercially available equipment (antisera, immunoassay kits, radioactive and other tracers...), qualitative and quantitative data about the monitoring programs performed by the Belgian Institute of Veterinary Inspection and Ministry of Agriculture, and chemical and physical data about the residues found in food products of animal origin.

3. REFERENCES

1. Official Journal of the European Communities, No. L 125/10 of 23.5.96, Council Directive 96/23/EC of 29 April 1996 on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC

2. Official Journal of the European Communities, No. L 125/3 of 23.5.96, Council Directive 96/22/EC of 29 April 1996 concerning the prohibition on the use in stock farming of certain substances having a hormonal or thyrostatic action and of beta-agonists, and repealing Directives 81/602/EEC, 88/146/EEC and 88/229/EEC

3. Official Journal of the European Communities, No. L 224/1 of 18.8.90, Council Regulation (EEC) No 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin

4. Official Journal of the European Communities, No. L 118/64 of 14.5.93, Commission Decision 93/256/EC of 14 April 1993 laying down the methods to be used for detecting residues of substances having a hormonal or a thyrostatic action

5. Official Journal of the European Communities, No. L 118/75 of 14.5.93, Commission Decision 93/257/EC of 15 April 1993 laying down the reference methods and the list of national reference laboratories for detecting residues

NP/DD/04

NORMS, REGULATIONS, DIRECTIVES IN ANIMAL PRODUCTION CHAINS: QUALITATIVE AND ENVIRONMENTAL IMPACTS

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EXECUTIVE SUMMARY

European agriculture undergoes deep mutations that are now well known. Current context obliges both to control production volumes and to reduce costs while improving product quality to maintain their market share and preserve environment, and to occupy and valorize available rural space. We must leave pernicious chains that are developing surpluses of production, qualities diminution, activities concentration, pollution, rural exodus (Béranger, 1992).

The production reduction measures that the states took following the CAP reform (Common Agricultural Policy) as well as the new proposals may promote disintensification. Redeployments to other less polluting systems, which must so least as possible affect farmer financial margins, have been facilitated by regulations on milk quotas and dairy cows subsidies. However, it's important to avoid that resigned farmers envisage it only through subsidies to compensate income losses. It's therefore a question of reasoning these modifications and incentives in terms of farming systems reorientation towards production methods better integrating environmental preoccupations and assuring systems durability.

Durable development notion appeared in February 1992 in the European policy texts at the time of the European Union Treaty signature, and, at the international level, in June 1992, following the Rio conference. The CAP reform in 1992 allowed integrating this notion in the European agricultural policy and, consequently, each member state had to reason its agriculture in durable development terms.

In a durable development optic, it's important that each state has a lot of levers (norms, regulations, directives, subsidies) which will allow, within each path, to control or orientate impact of each production system as well as on products quality than on local environment and, a fortiori, on planetary ecosystem.

To make the norms coherent and to imply them in a quality and durable development context, it's also important they are reflected on the totality of each path. By animal production path, we mean interconnection from upstream towards downstream of primary (production), secondary (transformation) and tertiary (marketing) sectors.

Thanks to the Belgian and European legislation's inventory that has been realized and organized in paths (http://www.cragx.fgov.be:83), we have been able to retrace the evolution of Belgian and European legislation about animal production paths, essentially bovine, porcine and poultry. It passes from 4 texts before 1900 to more than 800 after 1995, what proves upheavals and modifications it has undergone in the course of time and mainly since 1985, the date of the European Unique Act

signature and of the production control and environment protection policy. Indeed, until then, one objective was aimed: the maximal agricultural production necessary to feed a constantly growing population. Progressively, with mechanization and nitrogenous fertilizers appearance on the market, soil drainage, etc., production considerably increased. Gradually, we noticed negative consequences it could have on the environment and, consequently, we legislate more and more to restrain these impacts.

Through a survey on today's agricultural regulation, it was possible to underline a lot of problems and to propose, with the help of preferential witnesses, some actions to take in order to orientate evolutions towards the durability.

Intensification of the dairy speculation up to in years 85 and massive use of nitrogenous inputs has resulted in increasing nitrogen content of sewages but also in concentrating production in some regions of the country, entailing a rupture in the potential of recycling organic matters and elements such as nitrogen or phosphorus. In this regard, a study has been led in collaboration with the APEDB (Association des Eleveurs et Détenteurs de Bétail de Herve) in order to make a first report of the situation.

So, in the Grazier Region of Liège dairy farms, we could underline diversity of situations relating to efficiency of nitrogen use and speculation profitability.

Four categories have been identified, between which nitrogenous efficiency varies from 0.21 to 0.35 for a gross profit margin going from 65 000 FB by hectare to more than 100 000.

It was very interesting to observe that the most efficient farms were not necessarily the less profitable ones but that all situations exist in practice: it's therefore possible to conciliate a good efficiency and a good gross profit margin.

Giving farmer a better sense of responsibility and advising him judiciously, it's therefore possible to better reason nitrogen utilization, while not decreasing jointly his gross profit margin.

Others reflections were led in the porcine sector facing to speculation extension in Wallonie. Indeed reception capacity exists in agricultural Walloon regions but we have to reason this implantation to avoid well known in the North of the country drifts and to allow a more durable production in the South.

The CESRW (Conseil économique et social de la Région wallonne) has for example looked into the problem and suggests revising exploitations classification. Walloon

Region itself envisages to modify breeding buildings construction and exploitation rules, that within regional development new regulation.

Pig has therefore a beautiful future ahead in south Belgium and why not in the open air?

Now, concerning poultry, among diversity of poultry production systems, some have popular preference due to their positive image (animal welfare, environmental protection...) and from their more durable character. It's so for label poultry. a "Walloon quality label - flesh chicken"; has indeed been instituted that doesn't find its equivalent in Flanders and testifies to a Walloon agriculture more oriented to durability, because, if this breeding type image is positive, it seems to be similarly for environmental impact and products organoleptic quality. These aspects have however still to be developed in order to determine precise differentiation criteria.

Different solutions are proposed in order to guarantee some durability to our agriculture.

Durability by quality is a first example: the ISO is an increasingly sought-after approval and gives guarantees as for manufacturing process quality. It can besides well be applied to agricultural paths, it's for instance the case of animal food.

Another kind of quality is guarantee offered by label products whose poultry case is a particularly concrete example, due to existence in Belgium of a very well developed label Walloon chain.

Besides this particular production type it exists a whole series of quality traditional paths. More and more private initiatives starts via breeders associations, groupings, cooperatives particularly credible because will emanates from circle itself and not necessarily from legislator or politics.

In order to gradually install a durable agriculture, a system the legislator frequently uses is concession of subsidies to products, to animals, to hectare. Some subsidies have perverse effects, others must be encouraged, as for instance breeding connection to grass or inputs taxation.

As regards Belgian animal legislation oriented towards this durable concept, it would be a pity to omit animal identification system called SANITEL, originally conceived in a sanitary purpose and serving increasingly to support a policy of durability. This system allows indeed an animal follow-up in farms but also increasingly a tracability of animal products. This initiative really goes towards an agricultural systems durability allowing a better knowledge of animals, their course and their products

course increasingly sought-after by a consumer destabilized by the mad cow crisis. An efficient system would allow to restore consumer confidence in its local agriculture. About it, many initiatives are taken by public authorities in order to increase credibility of the instituted system, for instance utilization of electronic identification with a transponder.

In conclusion, we would like to insist more particularly on two points:

- Necessity of a better information about the existent legislation. Indeed, people rather badly know laws and regulations as well as context in which they are established.

  We must therefore inform the sector by distributing systematically to concerned people a popularization text of the legislation when it appears. A solution in order to attain this objective would be, why not, the regular update of the realized Internet site, what would allow keeping a maximum of people informed of this legislation evolution.

- Necessity of a complementary step. Indeed, we have seen that used means to make agriculture more durable are mainly vertical, in other words imposed by the legislator. However, it's important to also give to the breeder himself opportunity to evaluate and demonstrate its work quality and to reason in terms of durability ("bottom up approach"). It will be necessary to give him means to make it, notably by a technical and scientific support, and therefore a contribution of reasoned and durable agriculture indicators. Giving breeders a better sense of responsibility and informing them about the essential role they play for the environment, lifestyle and society, will be the most efficient means to make our agriculture more durable, both for men and environment.

NP/DD/05

CHECKING THE AUTHENTICITY OF FOOD AND FEED PRODUCTS HAVING SPECIFIC QUALITY

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SUMMARY

The objective of this study is to identify the various official or private regulatory frameworks that exist in Belgium, make an inventory of the corresponding special quality products and identify, with respect to each, the existing or potential analytical criteria by which their conformity with the specifications, and hence their authenticity, may be checked. Analytical methods already in use or which show potential (in Belgium or abroad) in the area of authentication are also listed and evaluated. As well as this paper, an Internet site for special quality Belgian products has also been set up. This can be reached at: http://www.cragx.fgov.be

Quality has many, sometimes contradictory facets: it is a matter of taste, cooking, hygiene (microbiology) and also of industry, regulations, business, as well as ethics and the environment ... The formula Q=3S+H+R+P neatly expresses the concept of quality. The fact is that everyone agrees that the quality of a foodstuff should meet certain criteria: **Safety** and **Health** are two requirements that must be fulfilled. These are indeed the subject of detailed legislation. Then there are **Satisfaction** and **Service**. As well as these four, there are **Regularity** and, lastly, **Perfection**, a more subjective concept.

Special quality products claim to be different chiefly in terms of Satisfaction, Service and Perfection. By laying down further rules in addition to those provided by law (e.g. size of farms in the case of broiler chickens), stricter standards than required by law (e.g. ban on using bone meal in feed for quality-label poultry) or extra inspections, the certification systems for special quality products are aimed at guaranteeing conformity and thus reassuring consumers shaken by hormone and "mad cow" scandals and unconvinced that genetically modified products on the market are safe.

The future looks bright for special quality products. They meet consumers' new expectations and are in line with developing sustainable agriculture in a number of ways:

1) food safety

2) focusing on protecting the environment

3) developing poor areas

4) preserving jobs in rural areas, improving farm incomes and helping rural populations stay on the land

5) palatability and nutritional quality to satisfy the most demanding desires and tastes

Legislation to protect these sectors has also been drafted and implemented, in some cases coupled with financial aid. Lastly, the supermarket chains are showing considerable interest.

According to an EEC study, special quality certified products (QCP) made up over 7.5% of the total EEC food market in 1991, just under 40 billion ECU. The study also outlines a plausible scenario in which the market share held by these products could increase to 50% by the year 2000, thus accounting for over 11% of the total EU foodmarket. Such development would be mainly due to strong growth in organic farming and ecologically based production (integrated production), the development of fresh meat certification procedures (certified special quality) and other similarly certified products, while alcoholic beverages and fat products (goose and duck foie gras, etc.), which are already well established, would stay as they are.

At European level, regulations relating to special quality products are proving successful. There are already around 422 Appellations d'Origine Protégées (protected indications of origin) and Indications Géographiques Protégées (protected geographical indications). In the case of Spécialités Traditionnelles Garanties (guaranteed traditional specialities), the legislation is more recent and the first applications have only just been filed.

In Belgium, the recent development of special quality products has been primarily influenced by the emergence of new European legislation with which Belgium has had to comply. At the same time, the Walloon Region has devised a legislative framework of its own: Walloon Quality Labels. This is the only identification used in agriculture. Similarly, a specific distinctive symbol has been introduced at federal level for integrated pomaceous fruit production.

Special quality symbols have a high profile in the animal production sector. They are a response to the need to restore consumer confidence and brand image.

Official and private distinctive symbols exist side by side (though this is not confined to Belgium).

Another point to be noted is that organic farming legislation currently only covers crop growing. Legislation on products of animal origin ought nevertheless to be passed shortly at European level. It may be noted that approved bodies such as Ecocert already control this kind of product. Finally, we may note that this method of

production is particularly consistent, since the raw materials used must themselves be of organic origin.

Organic products undergo certification before being marketed. The aim of certification is to guarantee that the claims made about them are genuine on the basis of a detailed set of standards. Certification has three components: a voluntary undertaking by the farmer, the specifications and the certification procedure.

The production rules set out in the specifications directly or indirectly affect the quality of the end product. In the case of animal products, these usually include the origin of the animals, links with the soil, the characteristics of the outdoor environment, buildings, feed, permitted or prohibited medication, conditions of transport and slaughter, and so on.

In the case of crops, emphasis is mainly on pesticides and fertilizers, with lists of permitted and prohibited products.

The certification procedure comprises three levels:

1) Certification proper, by an independent certification board, leading to the granting of a certificate to the farmer or a warranty to the product;

2) Inspection (visual, administrative or analytical) by an approved inspection body;

3) Authentication by an approved laboratory.

This comprises an analytical system for checking that the end products (e.g. meat) or raw materials (e.g. cattle feed) comply with special quality standards laid down in specifications.

While there are still considerable problems in the area of analytical inspection (detecting hormones, detecting antibiotics, detecting bone meal, detecting certain kinds of adulteration, traceability and use of genetically modified organisms, etc.) in respect of which certifying bodies are demanding methodological support, many techniques are being developed in an attempt to provide solutions. We therefore propose to develop new analytical methods to enable the competent bodies to check compliance with the additional standards more easily in connection with certified quality products.

As part of the pilot project following on from this inventory (" Development of analytical systems for checking the authenticity of certified meat "), the Agricultural Product Quality Department at the Gembloux Agricultural Research Center will investigate the most promising approaches to authenticating certified meat

(molecular biology techniques by which the genetic origin of products can be determined, near infrared spectrometry making it easier to recognise "certified" and "ordinary" chicken portions) and animal feed (near infrared spectrometry combined with other, complementary analytical methods) and also detection of antibiotics (microbiological screening combined with HPLC). New developments, which will undoubtedly emerge in this field, will also come under the Department's scrutiny.

Another finding from this inventory was that there is a wide range of distinctive symbols, corresponding to a similar number of different certification systems. This leads to different standards and lack of clarity for the consumer. We think it would be advisable:

1) to harmonise regulations on certification procedures, general conditions of approval of certifying bodies, procedures for laying down standards, inspection procedures (this proposal has in fact already been partly fulfilled in the case of recognised products at European level by the introduction of series 45000 standards);

2) to increase collaboration between certifying bodies and official inspectorates for even wider product cover.

In conclusion, the interest in specific quality products is justified by the response from consumers (which is already helping to develop production) and by the answers they offer to the aims of sustainable agriculture. Such products are a major avenue of economic development and therefore deserve specific attention. There nevertheless still appear to be many problems as regards analytical inspection. If no solution is found, the entire credibility of these products could be at stake.

NP/DD/06

RECOMMENDATIONS, PRE-NORMATIVE RESEARCH AND NORMS FOR A SUSTAINABLE AGRO-FOOD PRODUCTION CHAIN

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SUMMARY

The purpose of this study is to identify legislative weaknesses and shortcomings and to make some recommendations and propose some orientations towards a more sustainable system of agriculture.

To this end, an inventory of European, federal and regional legislation on agricultural practices and the marketing of inputs and crop-derived products has been drawn up. The international agreements governing commercial relations in agriculture complete the framework.

In the area of cultural practices, the specific standards affecting the sustainability of agriculture have been extracted from the legal texts in respect of each stage of the process. Considerable differences in the amount of legislation devoted to each stage have thus been identified. Agricultural land management is only subject to measures concerned with taking land out of use (set-aside and afforestation), whereas cultivation of the land is not regulated. Fertiliser use and in particular pesticide application, on the other hand, are governed by a more substantial body of regulations. The rules and recommendations made to control fertiliser use are supplemented by those deriving from water protection or waste management legislation. The rules governing pesticide application are either commercial or public health based.

As regards fertiliser use, rules and recommendations have been placed in the context of cultivation methods in order to identify shortcomings and weaknesses. In some cases, the way they are put into practice has been assessed. Gaps between the facts and the recommendations have thus been uncovered. These highlight particular areas where information, incentives and innovation are especially called for in order to attain the objectives underlying the regulations. Lastly, specific proposals and approaches to amending the rules have been put forward.

As far as plant protection products are concerned, European policy is first reviewed to show its influence on national legislation in the different member states. Before moving on to legislation specific to this country, the effect of pesticides on health, on the components of the environment and on certain living organisms is briefly described. Among other things, this reveals worrying problems of infringement of European drinking water standards. The challenging of these standards by some member states is also mentioned. A summary of pesticide sales for agricultural use is supplied to provide an assessment of quantities used in this country.

Next, the approval process set up in this country is explained and its weaknesses discussed. A need for other measures to complement this system is thus revealed. The regulations specific to this country are then examined. One chapter is devoted to a detailed discussion of the legislation on mandatory sprayer control, including a review of the objectives and implementation. The importance of the educational aspect of such controls is clearly evident, as is the desire for as few constraints on the user as possible. The positive results of plant protection product packaging waste collection, first started two years ago, are then described.

The remainder of the study is devoted to the principles of integrated control, with an inventory of the various warning systems available to Belgian farmers and the incentives introduced to encourage the use of such systems, which lead to a reduction in pesticide use. The industry's expectations of such a mechanism are summed up in one section.

Integrated pomaceous fruit production is the subject of the following chapter. Apples and pears are in fact the only crops to enjoy legal recognition of the integrated production system. The philosophy behind organic farming is then described, together with the way it is organised on our markets, the subsidies available and its importance within the country.

Pesticide reduction programmes in Sweden and the Netherlands are then used as examples to demonstrate the full importance of introducing co-ordinated initiatives through global programmes, something lacking in Belgium.

At the same time as this survey of regulations, crop-growing practices themselves have been studied in detail.

Experience and the results of research conducted by the Gembloux Agricultural Research Centre, among others, have identified common practice at each stage in the crop growing chain. The techniques, choices and habits which, due to their environmental or economic consequences, threaten the future of agriculture have been highlighted. In some cases, this means no more than lack of familiarity with a technique once forgotten and now revived (sowing small seeds on fallow land) or negligence (proper adjustment of spreaders). In other cases, the market-led pace of change in farming practices is creating gaps in the knowledge of some parameters essential for success (influence of the cropping history on each type of crop, varietal characteristics, soil quality), catching agricultural machinery development unawares (spreaders unsuited to the various organic materials on sale) or leading to uncertainties as to the repercussions of the new directions on health and the environment (recycling of certain waste through agriculture). Lastly, some

established practices cause environmental damage (intensive soil tillage, land left bare in the off-season, etc.).

The solutions and options put forward are listed. From supervision of farmers to stepping up research in fast-changing areas, these also include replacing certain established practices. As regards the latter, the options suggested in many cases still contain grey areas that need to be clarified and possible contraindications that need to be checked; these have been highlighted in the discussion.

Finally, a summary of the proposals for both amendment of regulations and changes in farming practices is provided in the study conclusions.

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AGRONOMICAL, ECONOMICAL AND SOCIOLOGICAL NORMS AND INDICATORS FOR SUSTAINABLE FARMING SYSTEMS

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EXECUTIVE SUMMARY

The feasibility and interest of models of sustainable agricultural systems are evaluated in three steps. First, foreign agricultural systems are studied according to the systemic approach. In all, six categories of agricultural systems are compared:

(1) conventional agriculture,
(2) integrated fight,
(3) agriculture without pesticides,
(4) integrated agriculture,
(5) biodynamic agriculture, and
(6) sustainable agriculture.

Constituents of agricultural systems are put forward, notably their objectives and sustainability indicators. Within each category of agricultural system, agricultural practices and sustainability indicators and norms may also vary with the context and the objectives of its promoters.

Secondly, mathematical models of sustainable agricultural systems are analysed. Few models take account of agronomic, economic and environmental results at the same time. Moreover, existing models represent a limited number of objectives. Producer behaviour against risk and uncertainty about events are rarely considered. Finally, those models stay static and give optimal results, without explaining the way to reach it.

Thirdly, thanks to the study of the agricultural systems and the existing mathematical models, modelisation feasibility of a farm level Belgian sustainable agricultural system is evaluated. For a decision support model, goal programming and interactive programming seem the most interesting. In other respects, expert system is recommended, for it makes the decision support model user-friendlier.

In the end, the understanding of agricultural systems facilitated the integration of sustainability indicators and norms in accordance with a systemic approach. To determine the Belgian sustainable agricultural system, the same methodology as for the study of foreign sustainable agricultural systems is proposed.

NP/DD/12-14

INTEGRATION OF ENVIRONMENTAL ISSUES AND HEALTH ASPECTS IN NORMALISATION OF FOOD PRODUCTS

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EXECUTIVE SUMMARY

The Flemish Institute for Technological Research (VITO, co-ordinator), the Centre for Education and Training for the Food Industry (COOVI-CERIA) and the Human Ecology Department of the Free University of Brussels (VUB) joined forces in this research project which is entitled "Feasibility of integrating environmental and health issues in food regulation". The project started early March `97 and was finalised by the end of February `98.

The most prominent goal of the study was to make an extensive **inventory** of the knowledge, which is available on the environmental impacts associated with food chains. Points of interest in this inventory were regulation (on food and environmental issues), existing schemes for the award of quality- and bioabels, experience with integrated chain assessments (LCA and HACCP), and an assessment of the terminology and the initiatives in the area of sustainable food production and consumption.

Concerning the **regulation and the normalisation of food products** in the field of health, labelling and environmental aspects, the Belgian food legislation has been thoroughly studied. First, the conception has been explained, with a review of the different types of regulations, namely, basic regulations, general or horizontal regulations, specific or vertical regulations and economic provisions. All the references from these regulations dealing with health, labelling and environmental aspects of food products have been registered in a detailed inventory. For that purpose, selection criteria for restraining those three types of aspects were defined on beforehand. The resulting inventory is, as a complement to the Belgian Food Legislation, a skilful tool for everybody who has to consult the texts on food legislation.

The assessment of legal requirements indicated that more and more attention is paid to food hygiene in general, in particular on meat.

The microbiological normalisation of food needs, in our opinion, an extension to the different groups of food products. Concerning the additives, Belgium is following the new EC-directives in this field; the texts already translated in the national law are nevertheless, qua lay-out, very unpractical to use, in comparison with the previous lists. In the field of heavy metals and pesticides, the existing norms do offer sufficient protection. An extension of the normalisation of toxins from microbial origin to spices and dried fruit seems to be necessary. Normalisation of dioxins forces itself also. Furthermore, the Belgian legislation offers guarantees about polycyclic aromatic

hydrocarbons, migration residues of packing material, irradiation, residues of veterinary drugs, administration of hormones.

Both horizontal as well as vertical labelling regulations exist for (prepacked) food products. Providing clear information for the consumer in this field is, in our opinion, highly necessary. Here, the government plays an important role. With regard to quality and ecolabelling systems, for which, at the moment, there exist no legal criteria, except for a few ones, there is a high need to a normalisation system.

Further on, food legislation in Germany and the Netherlands has been compared, up to a certain level, with the one in Belgium. For one type of product, namely milk, a comparison of normalisation concerning health, labelling and environmental aspects was made for the three national legislation's.

Finally, the recent developments in the field of genetically modified organisms (GMO) and Novel Foods have been outlined.

Next to the food regulation, also the **environmental regulation** of relevance to some typical branches of the food industry (meat and meat products, fruit and vegetables, dairy and beer) in Flanders, Wallonia and the Brussels Region has been investigated.

There exist significant differences in regulation in those three Regions, e.g. in the area of granting permits for certain activities. Since the Decree on Environmental Permits came into force in Flanders in 1991, different permits (e.g. the exploitation permit, the emission permit and the construction permit) have been integrated into one permit: the so-called Environmental Permit. In the Brussels Region, a similar kind of environmental permit exists, which incorporates the former emission permit. Thus far, this type of integration did not come into force in Wallonia yet. A draft decree of the Walloon Minster Lutgen, intending to group different permits, has been adopted on July 24 of 1997. This illustrates that the Belgian legislative authorities work on a harmonised environmental legislation in the three Regions.

The list of activities, which require an exploitation permit as, requested by the Flemish "Vlarem I" regulation has 52 categories, having numerous subcategories. The list which is used by the Brussels Region has 169 categories which are not subdivided any further. The four types of activities, which have been used as a reference in this assessment, are mentioned in both lists. One remark has to be made: the potato processing industry is not considered as being a separate category in the list of the Brussels Region.

In Flanders, neither the production of meat and meat products, fruit and vegetables, dairy and beer need to submit a EIA. In Brussels, activities of category IA need to submit a so-called effect-assessment, category IB type of activities only a effect-report. Class II type of activities are not subjected to any type of effect study. The types of activities considered here fall within the two last mentioned categories.

Finally, it has to be mentioned that, since the implementation of the decree on environmental management in companies (`95), specific categories of companies have to appoint an environmental co-ordinator. In the Brussels Region and in Wallonia, there is no such requirement. Thus, harmonisation in this area between the three Regions is required.

Furthermore, an inventory is made of **quality- and biolabels** for food products. To make this inventory manageable, four categories of food products have been assessed: diary products, meat, fruit and vegetables.

The inventory and assessment of existing labelling schemes shows that only few bio- and quality labels are officially recognised by the Belgian authorities. Many labels are based upon initiatives from vegetable- and fruit producers, cattle breeders, hypermarket chains, federations within the area of agriculture and other movements. They work with their own award criteria and rules, which are not officially approved by the Belgian authorities. Especially in the category "meat", there is a lot of confusion: there exist at least thirteen different bio- and quality labels for meat, each of them based upon different regulations and award criteria. In addition, there are (often on a local level) different brand names which are considered by the consumer as a quality labels, whereas these labels only give a new name to existing labels. Statements that meat does not contain any growth hormones are superfluous and misleading to consumers, since according to the Belgian law, the use of hormones is forbidden in cattle breeding: all meat that is put onto the Belgian market has to be free of hormone residues.

It can be concluded that there is a need for harmonisation at the level of rules and criteria for the award of quality- and biolabels. Exemptions where organisations (e.g. Biogarantie) want to apply stricter rules should be possible, especially when these initiatives have a national or international dimension and when the consumer is well informed about the added value of the label. Starting point for such exemptions however should always be the award criteria, which are approved by the authorities.

With regard to monitoring and control on the use of quality and biolabels there is no uniform procedure. Therefore, it is recommended to incorporate in regulations the

need to perform controls by independent organisations, which are certified in Belgium or Europe.

The award of quality- and biolabels can only be successful if the consumer is sufficiently informed about the contents and scope of the label: the added value of a labelled product next to an alternative product which is not labelled has to be clarified.

Within the framework of the study an assessment has been made of two tools which are used more and more frequently in the food sector for **integrated chain assessments**: LCA or life cycle assessment (focusing on environmental impacts throughout the product chain) and HACCP or Hazard Analysis Critical Control Points (focusing on biological, chemical and physical contamination in the food chain).

Within the framework of the research project, the **environmental impacts** associated with the various parts of the food chain have been assessed: production of primary food products (agriculture, farming...), processing, transport, distribution and consumption.

At the level of the primary production, the environmental bottlenecks are associated with manure production and disposal, the dispersion of toxic materials (pesticides, heavy metals...) and soil erosion.

Assessment of some branches of the processing industries highlighted that main problem areas are located at the level of the consumption of energy and water, the production of waste (organic waste, packaging waste...), the emission of odour and noise. A reduction of these impacts is expected from the further implementation of clean technologies (Best Available Technologies, Clean Technologies) and environmental management systems (EMAS).

Environmental impacts associated with transport and distribution are related to the use of packaging (waste) and the consumption of energy (transport and cooling). At final consumption, bottlenecks are food storage (cooled, frozen) and food preparation (energy, water, waste of packaging and non-consumable food portions).

In the final part of this study, a detailed assessment has been made of the terminology and initiatives in the area of **sustainable development**. Both Agenda 21 and the Fifth Framework Programme of the EC describe a general strategy for sustainable development. The focus in both initiatives lies on the common responsibility through more commitment of different economic actors and societal movements, regular review and monitoring. Both initiatives develop ideas in the area of sustainable food production and consumption and on sustainable agriculture. The

literature review showed that there is a need for a better-defined and more consistent terminology with regard to sustainability. Linked with this, sustainability indicators need to be developed which do not only take into account environmental issues, but which incorporate social and economical considerations as well.

In Belgium, a phased approach with regard to sustainable development is initiated. Belgium has engaged itself to test sustainability indicators at a local level.

Next to the detailed assessment of the terminology and the initiatives in the area of sustainable development an overview has been made of the international organisations which are active in the area of sustainable food production and consumption.

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SUBSTANCES RESPONSIBLE FOR FOOD INTOLERANCES: STATE OF NORMALIZATION

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EXECUTIVE SUMMARY

The present research project takes part in a sustainable development program about food quality. It is precisely aimed at investigating substances recognised to provoke adverse reactions to food. This topic is partly included in the framework of the European project eFID (European Food Intolerance Databank).

In addition to allergens studied by the eFID program as defined above, the research team n°1 participating to the present work (Biochemistry and general Physiology Laboratory headed by Professor G. Dandrifosse, University of Liege, Belgium), proposed to enlarge the aforementioned databank to biogenic amines and benzoates, also known to induce health troubles when ingested under certain circumstances.

Among biogenic amines, the most common diamines (cadaverine and putrescine) and monoamines (histamine, tyramine and tryptamine) are generally formed by microbial decarboxylation (from lysine, ornithine, histidine, tyrosine and tryptophan respectively). When occurring in high concentrations in food and beverages (principally fermented foodstuffs), they present a potential health risk for the consumers. While the histamine poisoning is the best documented case of biogenic amine-induced illness, several other biogenic amines have various toxicological effects, such as hypertensive crises provoked by ingested tyramine in patients under treatment with monoamine oxidase drugs (or anti-depressive drugs).

Among other substances promoting food intolerance, sodium benzoate and benzoic acid are anti-microbial, effective against bacteria and yeast. Benzoates are widely used as preservatives in foods and drugs and are often reported to cause skin reactions (urticaria and angineurotic oedema) mostly in patients with aspirin hypersensitivity (ASA patients). Controlled studies have also demonstrated that benzoates can be involved in atopic dermatitis, as well as in asthmatic or even in anaphylactic reactions. Furthermore, it has been estimated that ingestion of benzoic acid may exacerbate the skin lesions in children with atopic dermatitis. As a result, benzoates are the first most frequent cause among the preservatives implicated in drug adverse reactions in the UK. Therefore, it is of crucial interest to evaluate whether the standardisation of benzoate concentrations in foodstuffs meets the safety requirements for any consumer.

Indeed, beside known added quantities of preservative agents, food items could bear natural non negligible amounts of these substances.

The first objective of this part of the work was to evaluate to what extend biogenic amines and benzoates are standardised into foodstuffs consumed in Belgium. This part of the work takes place in a European project planed to construct a databank listing foodstuffs considered to be quite safe for any consumer, a program called eFID (for European Food Intolerance Databank), conducted in Belgium by G. Dufourny (supervisor of the team n°2 participating to the present program).

Our research led us to the conclusion that concentration of biogenic amines are not regulated in Belgium yet, while the legislation applied to benzoates is not satisfying, because it does not take into account the natural occurrence of benzoates into food products.

Considering the generally admitted maximal value considered to be still safe for consumers (Halasz et al., 1994; Stratton et al., 1991)**, *we propose here to set a Belgian legal limit at the level of 200 mg/kg of solid food and 10 mg/L of alcoholic beverage for histamine, tryptamine and ß-phényléthylamine.***
The lack of Belgian standardisation for biogenic amines prompted us to begin our investigations of foodstuffs for these substances first.

A short training stay in the laboratory headed by the Professor Mariné-Font (Unitat de Nutricio i Bromatologia - CERTA, Facultat de Farmacia, Universitat de Barcelona, SP) allowed us to learn a technique to detect biogenic amines. Briefly, it consists in a high-performance liquid chromatographic (HPLC) method based on ion-paired chromatographic partition in a C18 reversed phase column, involving a postcolumn derivatization with a fluorescent product (o-phthalaldehyde). To optimally adapt this method to food products, we first focused our investigations on beers. We indeed logically could expect substantial amounts of biogenic amines in fermented beverages.

Belgian beers (+- 300 kinds) were analysed for their pH, alcoholic percentage (% vol.), as well as their concentration in 9 biogenic amines (tyramine, putrescine, cadaverine, histamine, agmatine, ß-phenylethylamine, tryptamine, spermine and spermidine).

All beer samples presented substantial amounts of agmatine and putrescine, also recognised as natural amines in beers because of their natural occurrence in the raw product used for fermentation (malt). Spontaneous fermented beers presented a high concentration in induced amines (especially, tryptamine and histamine) that most of time exceeded the maximal value considered to be safe for the consumers.

The data we collected here about biogenic concentrations in Belgian beers have to be included into the aforementioned eFID databank. Alternatively, they could be

considered for the establishment of a histamine database, like the German National Food Intolerance Databank (NFID), a pilot project recently developed in Germany (Diel et al., 1997).

Starting from the occurrence of bacterial induced amines in beers, we established a calculation formula for a quality index called biogenic amine index (BAI) reflecting the microbiological quality of this type of beverage.

Based on the formula proposed earlier to assess freshness quality of fish and meat products (Karmas and Mietz, 1977; Veciana-Nogues et al., 1997), we defined a BAI formula for beers, calculated as follows:

BAI (beers) = (TYR + HIS + CAD + PHE)/10

where () means amine concentration expressed as mg/L

Significance of the BAI value:

- it reflects a good microbiological quality of the beer when its value is lower than 2,

- it reflects a poor microbiological quality of beer - and then a high content in vasoactive amines (TYR and HIS, principally)- when its value is higher to 2.

A further microbiological analysis of beers enriched in tyramine and histamine (mostly, spontaneous fermented beers) should be conducted to identify origins of high levels of biogenic amines that could affect the health of the consumers. The same study should be applied to other foodstuffs assumed to bear high quantities of vasoactive amines, i.e. cheeses and fermented meat products. The whole work is a preliminary step towards proposals of methods aimed at the removal of amines from food products.

An important part of further investigations should also be devoted to systematically investigate food items for their natural content in benzoates, a prerequisite to address the question whether the current legislation regulating the use of these substances as preservation is in agreement with a safe consumption of the concerned foodstuffs.

Another part of the study achieved by group 2 aims at establishing a databank with the substances that could induce food intolerance such as proteins of milk, Soya, egg and wheat, the AZO colours and sulphur dioxides, lactose and gluten.

The tool that is necessary for establishing the databank is now operational.

When finalising the methodology in accordance with our team of specialists, it has been pointed out that for proteinic substances, AZO colours, lactose and gluten, 'free from" means without any presence and/or addition of the substance in the food product. For sulphites, "free from" means less than 10 mg / kg of the substance in the food product.

When collecting the data from the manufacturers, a justification of "free from" is required for the very substance in the food product, in the case of allergies (proteins from milk, Soya, egg and wheat) and in the case of cytotoxic reactions (gluten).

As for pseudo-allergies caused by AZO colours, sulphites and lactose intolerance, where the symptomatology is less severe, the justification is desirable.

Nevertheless because detection cannot be 100 % (0 ppm) sure, **our proposal is that the definition of the "free from" norm i.e. without any presence and/ or addition of the substance be accompanied by: "the quantities that are present in the food product cannot be detected by the most sensible techniques and following the most precise operative method approved by a committee of competent scientists."**

This proposal results from the careful examination of the directive 95/2/CE of February the 20 th 1995 about food additives other than dies and sweeteners.

At the present, the list of analytical techniques has been set up for AZO colours. On the base of this study, **we propose two techniques of chromatography, one normalised method AFNOR and one research method (HP) that consists in isolating and identifying water-soluble chemical dies. The choice of one of these methods will depend upon further lab tests and this achieved on different matrixes**.

On the other side, **selecting and listing** methods for analyse for the other substances is still to be done because this requires more than one year work.

Based on our work, **following information is to be formulated within the data accompanying the distribution of the "free from" lists** to the physicians, dieticians and chemists, i.e.:

1. The name of all components of the food products that could be suspected of containing the very substance;

2. Two warnings are to be made.

- The first one regards the labelling of food products that cannot be liable as far as ingredients such as milk, Soya, egg and wheat are concerned. For additives the risk could still be present: e.g. dies and their "carry-over" and "reverse carry-over" (TIMMERMAN G. et al., la Charte, 1995). The carry-over is authorised when the additive is allowed in one of the ingredients within the composed food. The reverse carry-over is also accepted when the food product is intended for elaborating composed food products wherein the very additive is allowed.

- The second warning consists in the analytical precision of the terminology: "without any presence and/or addition of the substance in the food product" which requires the following accompanying notice: "the quantities that are present in the food product are not to be detected by the most sensible techniques and following the most precise operating system approved by a committee of competent scientists".

At least, the process with a view to constitute an ASBL (association without pecuniary gain) together with public partners (Authorities and the Haute Ecole Lucia de Brouckère) and private partners (Fédération des Industries Alimentaires - Federation of Food Industries and Fédération des Distributeurs- Federation of Retailers) implied within the project will have constructive effect for its future.

We insist on the fact that the most scientific strictness when collecting the data is essential as far as food intolerance is concerned and the time we were allowed to for completing this task was too short to study and analyse all substances concerned by the project.

Partnership with industries will let us clarify the problem of the responsibility of the different partners. The manufacturers, the representatives of the databank and the representatives of the Art de Guérir (the art of Curing) are responsible for the information they give out and transmit. Those main objectives are the priorities for the continuation of the present work.

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NP/16/18

CHARACTERIZATION AND QUALITY CONTROL OF FOODSTUFFS BY MAGNETIC RESONANCE IMAGING (MRI)

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1. PROTOCOL FOR THE MEASUREMENT OF WATER ACTIVITY (a_w) BY LOW RESOLUTION NMR

Purpose. The purpose of our investigations has been to find an optimum NMR procedure (transverse relaxation times, T2) to characterise a_w in foodstuffs. We propose two different protocols for the measurement of T2, which differ by the type of mathematical processing (i.e. mono- and multi-exponential fitting) and by the information, which is obtained (i.e. water state in the *bound* or *free* compartment of the food system).

Materials and Methods. Samples of potato starch with different a_w were analysed by two distinct Carr-Purcell-Meiboom-Gill (CPMG) pulse sequences, which characterised water or oil decays. The cumulated relaxation curve of water and oil allowed a tri-exponential fitting for the T2 calculation. A mono-exponential fitting procedure has been tested in order to find a more convenient, fast and bust protocol for routine characterisation of a_w in food industry. For this purpose, the T2 has been measured with a pulse sequence that covered the first 10 ms of water decay.

Results. A distinction between water and fat protons during NMR measurement is essential, and it is the only possibility to find a relationship between the time constants of water and a_w, in complex systems. The multi -exponential fitting procedure reveals in the same time a complete information about the transverse relaxation of proton populations in the system, i.e. *bound* water, *free* water, oil. However, this method can be used with a high prudence when the food products contain other proton rich compounds, e.g. oil. The mono-exponential processing of the first 10 ms of water decay provides accurate results, which reflect with confidence the a_w of a food system. An automation of this technique should allow its implementation as a routine procedure for the assay of a_w in food industry.

Conclusion. Although the multi -exponential fitting procedure requires a large amount of data and is more time-consuming, it could be useful when a detailed information on the substrate state of hydration is required during food processing, i.e. the hydration of flour substrates during dough preparation. The mono-exponential protocol is faster and more practical, and provides a reliable characterisation of a_w even in the presence of oil. We have to emphasise that such a protocol is reproducible only for a certain type of product and for the same time domain parameters used in the acquisition.

2. THE QUANTIFICATION OF SOLID, WATER AND OIL IN FOOD SYSTEMS BY NMR METHODS

Purpose. Several low-resolution NMR methods are known in food industry to quantify the solid, water and oil in food products, but obvious problems still remain when their moisture content is greater than 5%. In the present study, some NMR protocols were proposed, which describe simultaneously the ratios solid/liquid or water/oil and the T2 relaxation times in foodstuffs when they are required for the measurement of a w. The novelty of the method developed in the University of Mons-Hainaut relies on the fact that it tries to surpass the actual constraints related to the quantity of water or oil in the food products, and imposed by the current techniques used in industry.

Materials and Methods. For the measurement of the solid/liquid ratio in samples containing only starch (or mousline) and water we used the free induction decay (FID) method. The FID+one spin echo (SE) and FID+CPMG sequences were used to quantify the percentages of solid, water and oil in the samples, which contained also oil (or margarine) in their composition. We replaced the "f-factor" (currently used to determine the amplitude of the FID signal at time 0) by an extrapolation function, which takes into account the T2 of solid and the type of signal decay. In all experimental situations, the results were calculated both by including or not the specific hydrogen proportion in the starch, water and oil molecules, and the data were compared.

Results. An accurate determination of the solid/liquid ratio has been possible in starch containing samples, excepting the ones with lower water content (16%), where a deviation from the real value was been observed. For complex model systems (starch, water, oil), the specific proton proportion improve the data at inferior water levels (< of = 20%). Higher water contents in these food models can cause errors in oil quantification.

Mousline powder contains in its grains about 16% "liquid-like" material, which can interfere with water signal and result in its overestimation. Furthermore, margarine contains about 9% solid at 25°C, which can contribute to the solid signal that belongs to the mousline powder and finally can result in its overestimation. A deviation from the real value has been obvious for the samples with lower water (63-70%) and margarine content (3%), while the percentage composition of each element has been generally better estimated by taking into account the correction factor for specific proton abundance.

Our results demonstrated that both NMR techniques (FID+SE and FID+CPMG) used in this study are adequate for the determination of the percentage composition in complex food systems, and their approach may depend on the type of information which is required. For example, the FID+CPMG procedure gives simultaneously the following information: the percentage content of solid, water (including bound and free water) and oil; the T2 of different water populations (e.g. solid-like, bound water, free water) and the one of oil. The automation of this technique will allow an on-line protocol for industry, and will be able to assay concomitantly the a w and the percentage content of different components in food systems.

Conclusion. The low-resolution NMR methods proposed here to quantify the solid, water and oil in food systems give accurate results even when moisture content is higher than 6%. If we add the complex information furnished by them, we can assert that our procedures have an obvious original contribution to the ones known up-to-date. The fact that NMR methodology is non-destructive, fast and reproducible represents another argument for their advantages above other conventional methods.

3. THE MEASUREMENT OF STARCH RETROGRADATION BY NMR METHODS

Purpose. The present study is supported by the importance of retrogradation for the quality of starch containing products, and by the theoretical and practical advantages afforded by NMR techniques. Due to the dynamic interaction between starch polymer and water molecules, the process of starch retrogradation can be monitored in time by 1H-NMR techniques. For this reason, the kinetics of potato starch retrogradation has been studied in the present work by low and high-resolution 1H NMR spectroscopy.

Materials and Methods. The transverse relaxation times (T2, mono- or multi -exponential) and solid/liquid ratio (FID technique), the proton exchange times (measured by Goldman-Shen sequence), and texture analysis on MR images were analysed as useful parameters that describe starch retrogradation in starch gels and potato.

Results. The evolution of the transverse relaxation times (T21 and T22; T23 can be also obtained by a tri-exponential processing) suggests that polymers' re-crystallisation is associated with a dynamic process of water binding - release, the dominating one being the water binding. Beside the time constants (T21, T22), A1 or A2 (the percentage amplitude of *bound* and *free* water) can add a supplementary

information about the gel's potential to hold water in its structure. This is an important attribute for the food quality and preservation.

The mono-exponential T2 decreased concomitantly with the progress of retrogradation, which suggests that water molecules have a lower mobility due to their holding in the re-crystallised polymer network. This procedure is accurate and fast, and can be implemented in industry for the assay of starch retrogradation. The NMR method does not need any special preparation of the sample, it is not destructive, and can give the result in a short time.

The solid content increased both in starch gels and potato concomitantly with starch retrogradation. The solid T2 exhibited the tendency to decrease, which could confirm the water holding and entrapping in re-crystallised molecules.

The Goldman-Shen experiment showed a constant increase of the bound exchangeable phase of water protons, which confirms our former investigations on transverse relaxation and solid/liquid ratio. A general tendency of the exchange times to increase in potato samples has been observed. This is a prove of the strong interaction between water protons and polymer molecule, which consequently facilitates the phenomenon of cross-relaxation.

Texture analysis reflects potato as a homogeneous paste interrupted by a dispersed distribution of some points of starch nucleation. Furthermore, the evolution of texture parameters seems to be a result of a decreased T2 and proton density. The method of texture analysis is promising and can find its applicability in a multitude of branches in food industry.

Conclusion. Different NMR methods may provide a particular information about starch retrogradation, and this depends on our requirements when we intend to analyse a food product, i.e. the state of water in the starch gel (e.g. its mobility), the percentage of bound water in its composition, the solid content which corresponds to the level of re-crystallized polymers.

4. NMR METHODS FOR LIPID ANALYSIS

Purpose. Low- and high-resolution 1H and 13 C NMR were used to analyse edible fats with different degrees of unsaturation, i.e. cocoa butter, animal fat, fish fat (hydrogenated fat), peanut oil, corn oil, sunflower oil. The purpose of our investigations has been to identify the more suitable NMR methods to analyse the physical and chemical properties of edible fats. Finally, we have analysed their usefulness as tests of authenticity for cocoa butters.

Materials and Methods. The samples were analysed by 1 H and 13 C NMR spectroscopy, and by low resolution NMR (T1 as an index of fats' unsaturation, and the solid fat content, SFC).

5. RESULTS / CONCLUSIONS

1 H NMR spectroscopy can be used to characterise the general structure of triacylglycerols. The 1H NMR spectra can furnish quantitative information, such as the molecular weight, the percentage hydrogen content of the fat molecule, the degree of unsaturation (iodine number), the average number of double bounds per fat molecule.

The **13 C NMR spectroscopy** is a useful tool for fat analysis. This method can provide a complex and accurate qualitative (chemical structure) and quantitative information (e.g., the relative proportion of unsaturated and saturated fatty acids, the average molecular mass, the degree of unsaturation). 13 C NMR spectroscopy can be used in fat industry both for the quality control analysis and as a test of authenticity.

The analysis of T1 as a parameter that describes the degree of unsaturation revealed its usefulness, excepting some fats such as the fish fat (hydrogenated fat). Therefore, the method can be used in fat industry for the fast measurement of the degree of unsaturation.

The **SFC** has been determined according to the methods described in literature, but some changes were made in the University of Mons-Hainaut in order to improve the actual methodology. These improvements refer to the NMR signal processing which results in an accurate measurement of SFC.

NMR methods for the test of cocoa butter authenticity. The new regulation adopted by the European Commission stipulates that 5% of chocolate can be

replaced by other tropical vegetable fats known as *cocoa butter equivalents* (CBE). Consequently, there is an urgent need at European level for methods capable to detect and quantify the CBE.

Our studies evidenced the following differences between cocoa butters and their equivalents: (i) the higher content of palmitic acid in CBE and in their mixtures with authentic cocoa butter; (ii) a lower stearic acid / palmitic acid ratio for CBE and their mixtures with cocoa butter; (iii) the 13 C spectra of some CBE (e.g. industrial cocoa butter substitute, illipé butter, and in the mixture of cocoa butter with palm oil) present supplementary signals assigned to diacylglycerols; (iv) the higher level of solids in some CBE (e.g. industrial cocoa butter substitute, illipé butter). However, our studies identified lower levels of solids in mixtures if we compare with their origin samples. This property is difficult to be used as a test of authenticity if we take into account the great variability of cocoa butters.

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STUDY OF *SALMONELLA* AND *CAMPYLOBACTER* CYCLES BY THE PRODUCTION OF BROILERS

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1. RATIONALE AND OBJECTIVES

The last decade is characterized by a dramatic increase of human cases of salmonellosis and campylobacteriosis in most Western countries. Figures of epidemiological research indicate that about 10 % of human salmonellosis and nearly all campylobacteriosis cases are caused by consumption of contaminated poultry meat. Together with the increase of these two important human food infections, an important persistence of these pathogens is noticed in the production chain of poultry meat. A control program the last decade is characterized by a dramatic increase of human cases of salmonellosis against the presence of *Salmonella* and *Campylobacter* in poultry products has to consider a reduction of the contamination in the whole production chain.

Salmonella and especially *S.* Enteritidis can infect hatching eggs and can so be transmitted from the parent poultry flock to the broiler. This is called vertical transmission. *Salmonella* can also be transmitted horizontally from the environment to the broiler. A policy, focusing only on the vertical transmission by clinical treatment and eradication of the *Salmonella* positive parent flocks, is not effective when the horizontal transmission is not controlled. Literature shows that risk factors for horizontal transmission are fluctuating over time and differ in function of the geographical location of the poultry farms. The research of this project aims to identify the most important *Salmonella* contamination routes in the broiler production chain and to formulate recommendations for an efficient combative program.

The situation differs for *Campylobacter* because this pathogen is only horizontally transmitted to the broilers. Only limited information is available in literature about the *Campylobacter* contamination routes to broilers and poultry meat products and this project aims to add to this knowledge.

Antibiotic resistance patterns often arise in human pathogens and constitute a considerable danger for public health. These resistance patterns are not well known for *Salmonella* and *Campylobacter* isolated from the production chain of poultry meat in Belgium.

2. TASK DESCRIPTION

To achieve the objectives of the project a multidisciplinary partnership has been established with the University of Ghent, Veterinarian faculty, Prof. Dr. L. De Zutter (RUG), the Agricultural research centre, Department of animal product quality, Dr. L. Herman and Dr. M. Heyndrickx (DVK-CLO), the Centre for research in veterinary medicine & agrochemistry, Dr. D. Vandekerckhove (CODA) and the UMC Sint-Pieter, Prof. Dr. J.-P. Butzler.

In the period from april 1998 tot march 2000, the *Salmonella* and *Campylobacter* contamination of 18 broiler flocks was followed in detail from the hatchery to the cooled carcasses in the slaughterhouse (see scheme).

Scheme of the production chain. The shaded zones are studied in this project by the indicated partners.

Production chain	*Parent flocks*	*Hatchery*	*Broiler farm*	*Slaughterhouse*
Sampling time		*day 22*	*6 weeks* *3 samplings*	*Transport* *Slaughtering process*
Sampling of		*one-day old chicks, environment*	*transport of chicks* *hygiene of rearing house, animals and environment during rearing*	*Transport containers* *Carcasses* *organs*
Partners		*DVK-CLO*		*RUG*

The *Salmonella* and *Campylobacter* isolates were collected, typed with molecular methods (DVK-CLO and RUG) and from a representative amount of strains, the antibiotic resistance profile was determined (UMC Sint Pieter). The obtained results were analysed by epidemiological statistical methods (CODA).

3. RESULTS

For the isolation of *Salmonella*, methods with an enrichment in Rappaport Vassiliadis (RV), in 'Diagnostic semi-solid *Salmonella* agar' (Diassalm) and in 'Modified Semi-solid Rappaport-Vassiliadis' media (MSRV) were compared. In total 3150 samples were analysed and a combination of selective enrichment in RV and Diassalm reached a sensitivity of more than 99 %.

Vertical transmission of *Salmonella* was demonstrated in 2 and maybe in 3 flocks. *S.* Enteritidis was isolated from broken egg shells in the hatchery and from the trayliners obtained after transport of one-day old chicks. These strains did not persist in the animals during rearing. *S.* Hadar, isolated from the trayliners did not persist in the broilers. From each of 4 houses a different serotype of *Salmonella* was isolated before arrival of the one-day old chicks: *S.* Virchow, *S.* Blockley, *S.* Hadar and *S.* Infantis. Only the *S.* Hadar and *S.* Blockley contaminations persisted by the animals during rearing. Overshoes were found positive in 10 of the 18 flocks during rearing and obtained a *Salmonella* positive status. The *Salmonella* status was most sensitively determined by the overshoe method. The results indicated, however, that different pairs of overshoes have to be taken to reach a representative sensitivity. In the farm environment, a high contamination level (11 of the 18 farms were positive) was found. In 4 flocks *Salmonella* was isolated from the feed: 2 *S.* Mbandaka, 1 *S.* Blockley and one non-typable isolate. *S.* Blockley persisted in the animals. The most important infection of the broiler flocks happened during the 2 first weeks of rearing, the amount of positive flocks decreased during further rearing. In 12 of the 18 flocks, antibiotics were administered to the broilers during the rearing period. Antibiotic usage influenced the amount of positive overshoes and caecal drops significantly ($p = 0,02$). The intake of movable material in the house after cleaning and disinfection was identified as most important factor for horizontal transmission of *Salmonella* during rearing ($p = 0,08$). The hygiene of the houses, other animals on the farm (inclusive domestic animals, insects, spins, rodents and birds), ditch water, puddles and other surfaces in the environment of the house did not function as a significant source for *Salmonella*. Also the amount of houses on the farm did not influence significantly the *Salmonella* infection of the animals.

With pulsed field gel electrophoresis (PFGE) several *Salmonella* serotypes could be further subdivided in genomic types. *S*. Mbandaka showed the greatest diversity in types; however, only one genomic type was sporadically found in the animals of 2 flocks. In 2 successive flocks in the same broiler house of a rearing farm, the same *S.* Hadar genomic type was dominantly found in the animals; this type can be considered as highly virulent in chickens. This type was already present in the

environment before arrival of the one-day-old chicks of the first followed flock. On this rearing farm, another *S*. Hadar genomic type was also present in the environment and in the house before arrival of the one-day-old chicks of the following flock, but this type was not found in the animals and is thus possibly not or less virulent for chickens. The *S*. Enteritidis isolates from the 2 hatcheries and 2 flocks belonged to 2 different genomic types. In one of these flocks, the same *S*. Enteritidis genomic type was isolated from the animals during the whole rearing period. In the other flock, firstly 2 other serotypes were isolated from the animals, and then after 6 weeks rearing a genetically changed *S*. Enteritidis strain (acquisition of a megaplasmid). In a flock followed on a circulation farm, the animals were contaminated with a dominant and a sporadic genomic type of both *S*. Blockley and a non-typeable serotype. The dominant types were transferred to the animals by unsufficient hygiene in the house before arrival of the one-day-old chicks, as well as to other houses by footwear. In 2 other flocks, transfer of the same genomic type of a certain serotype could also be demonstrated, respectively between houses and (probably) via feed to the animals.

At the slaughterhouse level, more *Salmonella* positive samples were isolated. No significant correlation was found between the infection during rearing and the contamination of the slaughtered carcasses. The positive faeces from the transport boxes are mostly derived from the transport boxes themselves. The identity of the slaughterhouse was identified as the most determinative factor for the contamination of the carcasses. Analysis indicated that neither the *Salmonella* status of the flock, nor the evisceration technique nor the time of slaughtering (slaughtered first or not) had a significant influence. Additional research showed that during slaughtering the feathers were found almost systematically contaminated with *Salmonella* even from flocks with a negative status. In some slaughterhouses the carcasses were already highly contaminated with *Salmonella* after plucking. Further processing resulted in a reduction of this contamination. From molecular typing with PFGE, it could be deduced that in only 5 flocks carcasses were contaminated with the same strain as isolated from the live animals. In one flock, carcasses were contaminated with a *S*. Hadar strain which was before only isolated from faeces of a dog on the rearing farm; possibly, unloading of the flock for slaughter can be responsible for this contamination.

No *Salmonella* isolate showed resistance for cefratoxin, ciprofloxacin and kanamycin. About 30% of the isolates were resistant for streptomycin, ampicillin, amoxillin and tetracyclin, about 12% for nalidixin acid and trimethoprim/sulfamethoxazole. Of the isolates, 42% were resistant for at least 1 antibiotic, 11% for 5 antibiotics. It was striking that all 49 *S.* Hadar strains were resistant for at least 2 antibiotics and most of these were resistant for 3 to 5 antibiotics.

Campylobacter was not found in the hatchery and by the one-day old chicks. Also no isolates were obtained from the rearing house before the arrival of the chicks. The *Campylobacter* status during rearing of the broilers was most sensitively determined by the analysis of caecal drops. The infection of broiler flocks increased continuously during the rearing time. Seven flocks in total were positive for *Campylobacter*; in all cases, *Campylobacter jejuni* was found, in only 1 flock also *Campylobacter coli* was found. In the environment of the house, *Campylobacter* was isolated in 11 flocks. The movable material and especially the footwear of the farmer were determined as significant risk factor (p = 0,036). The administration of antibiotics reduced the shedding of *Campylobacter* by positive animals. This effect was however not significant as it was for *Salmonella*.

From typing with PFGE and *fla*A-restriction analysis, it followed that each flock was contaminated with its own *C. jejuni* genomic type. *C. jejuni* is thus genetically a very heterogeneous species. The drinking water was frequently contaminated with the same genomic type as found in the animals, which can cause a further spread of the contamination in the flock. In 2 flocks, the animals were contaminated with several *C. jejuni* genomic types; in one of these flocks, 4 types succeeded each other during rearing. Transfer of the same genomic type from the environment or between houses (probably via footwear) could be demonstrated in several flocks.

After slaughtering, 12 flocks were positive for *Campylobacter* in the caecum content and 13 on the carcasses, which indicate an extra contamination during the slaughtering phase. This extra contamination was not correlated with the identity of the slaughterhouse and started by the transport of the animals. The contamination of the carcasses was clearly correlated with the contamination of the animals during rearing and not with the applied evisceration technique and with the time of slaughtering (slaughtered as first flock or not).

None of the 178 tested *Campylobacter* strains were resistant for amoxicillin/clavulaanzuur 2/1 and only 1 strain for gentamycin. For many antibiotics an intermediary resistance was established. About 27% of the strains were resistant for ciprofloxacin, nalidixin acid or tetracyclin, about 8.5% for erythromycin or ampicillin. Only 6% of the strains were resistant for all tested antibiotics, 13 % were resistant for only 1 antibiotic, 27% for 2, 10% for 3, 2 strains for 4 and 1 strain for 5 antibiotics.

4. CONCLUSIONS AND RECOMMENDATIONS

- For the isolation of *Salmonella* from poultry related samples, a combination of selective enrichment in RV and Diasalm reached a sensitivity of more than 99%.

- Vertical transmission of *Salmonella* still occurs, which indicates the importance of further efforts to control contamination in the parent flocks.

- Our results show clearly that there is a decrease of the relative importance of the first stages in production and an increase of the relative importance of the last stages (transport of broilers and slaughter). The extensive contamination during rearing is easily transferred from the environment to the broilers in the poultry house. It is important to correctly use the hygiene gate and to decontaminate the footwear.

- *Salmonella* contamination in a flock is most sensitively determined by the overshoe method. For this more than 2 pairs of overshoes has to be taken on different sampling times during rearing. Especially the use of antibiotics during rearing decreases the presence of *Salmonella* in the faeces and in the overshoes and has to be considered in control programs.

- The investigation of the presence or absence of *Salmonella* in certain samples and even serotyping are not always sufficient for the exact determination of contamination sources. In many cases, only molecular typing gives the necessary information for epidemiological links. Pulsed field gel electrophoresis with the use of the appropriate enzymes (*Xba*I and *Not*I) is a technique with sufficient resolution for the serotypes encountered in broilers amongst which the clonal serotype *S*. Enteritidis.

- Faeces from transportcontainers cannot be used to detect a *Salmonella* and *Campylobacter* contamination in flocks presented in the slaughterhouse. These faeces samples can be contaminated by unsufficiently cleaned and desinfected containers.

- No correlation exist between the status of the flocks and the contamination of the carcasses in the slaughterhouse. The identity of the slaughterhouse is of significant importance for the carcass contamination with *Salmonella*. An obvious contamination takes place during the first stage of the slaughtering process. The evisceration method and the time of slaughtering (slaughtered as first flock or not) do not have an important influence on the contamination of the final product.

- *Campylobacter* was not isolated from one-day old chicks and in the hatchery. The hygiene of the poultry house did not seem to play an important role in the contamination of the flock. *Campylobacter* is clearly transmitted to the animals during rearing from the environment with the footwear as most important vector. Also the drinking water is an important vector for further spreading of the contamination. This indicates the importance of an efficient hygiene gate and a correct disinfection of the footwear and of the drinking water.

- The presence of *Campylobacter* contamination in a flock is most sensitively investigated by the analysis of caecal drops. The amount of positive drops increases continuously during rearing of a positive flock. As a consequence, the best moment to determine the status of the flock is just before slaughtering. Each positive flock can be contaminated with a different *C. jejuni* genomic type; some flocks can be even contaminated with several (successive) types. The administration of antibiotics during rearing has a reducing effect on the presence of *Campylobacter* in caecal drops. This effect is not significant and more limited than found for *Salmonella.*

- The *Campylobacter* contamination during rearing is quite correlated with the contamination of the final product. This contrasts with the *Salmonella* results. The identity of the slaughterhouse is not a significant factor for the final carcass contamination with *Campylobacter*. Nevertheless, an extra contamination of the broilers is noticed during transport and during the slaughtering process, which indicates the importance of hygiene during these steps. The evisceration method and the time of slaughtering (slaughtered as first flock or not) do not have a significant influence on the contamination of the final product.

- Salmonella and Campylobacter isolates are both showing a considerable antibiotic resistance. Of the Salmonella strains 42% were resistant for at least 1 antibiotic, 11% of the strains were resistant for 5 antibiotics. The very high resistance of S. Hadar isolates is striking. Of the Campylobacter isolates 94% were resistant for at least 1 antibiotic, 2 strains were resistant for 4 and 1 strain for 5 antibiotics.

NP/42/22

DEVELOPMENT OF ANALYTICAL SURVEILLANCE OF CERTIFIED MEAT PRODUCTS

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EXECUTIVE SUMMARY

Certified products of a specific quality aim to meet higher standards than the minimum statutory requirements regarding health and composition. They are intended as a response to demand from consumers concerned over food safety issues. Such quality products, designed to be compatible with the environment (linking of products to the soil) and animal welfare, require specific guarantees if they are to be credible and viable. To achieve this, systems have to be set up to verify their conformity with the specific standards and also their labelling. Reliable analytical methods are an essential part of the control procedures.

Our research is focused on the "broiler", a product characterised by a variety of seals of approval and quality labels in Belgium and in Europe : quality labels (*Label de Qualité Wallon* - Walloon Quality Label, *Label Rouge* - Red Label in France, special labelling under regulation 91/1538/EEC), European regulations relating to labels of origin (*Appellations d'Origine Protégées*, protected indications of origin and *Indications Géographique Protégées*, protected geographical indications (regulations 92/2081/EEC and 93/2037/EEC)), Organic Farming (regulation 1999/1804/EEC, national legislation and private standards).

The Department QUALITY OF AGRICULTURAL PRODUCTS at the GEMBLOUX AGRICULTURAL RESEARCH CENTRE has joined forces with the UNIT OF ANIMAL HUSBANDRY at GEMBLOUX AGRICULTURAL UNIVERSITY to develop methods for authenticating meats and meat products using rapid analysis techniques (near infrared spectroscopy-NIRS) or molecular biology techniques. Verifying the conformity of poultry feed (no animal meal or antibiotics) by NIRS in conjunction with other analytical methods (HPLC) has also been studied.

During the time of the research, it was demonstrated that near infrared spectroscopy is an appropriate technique to distinguish slow-growing chickens from fast-growing ones. Discriminant models based on the analyses of chicken meat by NIRS were developed. With this fast, cheap and non-destructive technique, NIRS-models are able to authenticate a majority (> 80 %) of the samples, be it either on carcasses or on cut pieces. An animal experiment was also set up with the collaboration of the UNIT OF ANIMAL HUSBANDRY (Professor André Théwis - GEMBLOUX AGRICULTURAL UNIVERSITY) to evaluate the relation between feed composition and NIR spectra of meat. According to the results, it would be possible to detect more than 70 % of fraud cases (chickens bred in contradiction with the set of production rules including the use of slow-growing chicken strains together with a specific feeding). **Even if discriminant models developed are based on the spectra acquired on a**

population with a significant number of specimens (more than 150 chickens), it would be better to extend the databases and to test new algorithms (e. g. neural network analysis). A good optimisation of the technique should allow its routine application.

One of the conclusions of the project is that PCR-RFLP is not appropriate for detecting the components of a meat mixture although it is reported as such. The fact is that though the use of universal primers does have the theoretical advantage of not having to have preconceived ideas about the possible components of the blend, this technique in fact causes a clear bias in the detection of the species in the blend. For instance, in a turkey/chicken blend, without a doubt one of the most plausible frauds, it turned out that a 10% turkey content cannot be detected by the PCR-RFLP technique. Its presence only starts to be revealed from around 25%. This is explained by the fact that the primers, although regarded as universal, amplify the chicken in preference. In order to avoid such a bias phenomenon, the use of species-specific targets is recommended. Until now, we are able to detect adulterations of chicken meat with turkey meat at levels below one percent. **Nevertheless, the development of a strategy requiring a sole PCR to detect the various animal species in meat products remains very attractive. The use of new technologies (e.g. biochips) and specific capture probes internal to conserved regions would be a solution to the bias problems described before.**

With the aid of the AFLP technique (Amplified Fragment Length Polymorphism), we have succeeded in identifying two bands which in the population under study allow specimens from slow-growing strains to be distinguished from fast-growing strains. The two molecular determinants were isolated, cloned and sequenced : one is specific of slow-growing chickens (333 bp) and the second band is characteristic of fast-growing chickens (372 bp). The two identified markers are apparently correlated to growth rate. However, one may not exclude that these markers simply reflect a tight link with a particular breed used in the slow- or in the fast-growing strains. The exact meaning of these markers is unclear, the more as no homology could be found with known sequences (comparison with sequences registered in international sequence libraries) and hereby for instance give some indications of a possible link to growth. This statement does in no way diminish the usefulness of these markers (especially in so far as verifications for use of JA strain as a slow-growing strain are concerned) but could restrict their domain of application (e.g. valorisation limited to slow-growing strains resulting from a specific breed in the ascendants…).

The choice of AFLP as a technique for investigating the polymorphism of DNA was based on the fact that it does not need any prior knowledge of the genome and is

reproducible. Results to date allow us to envisage with optimism the possibility of distinguishing between slow-growing and fast-growing chicken strains according to their molecular determinants. AFLP nevertheless appears to be suitable as an investigative technique for DNA, though we feel it is too cumbersome to use for routine analyses (it can take two days to get a result). **It seems an evidence that development and validation on a representative set of a fast PCR test (results within one day) based on the determinants identified by AFLP has to be considered. A test of this kind could be routinely used by a testing laboratory but requires beforehand that the exact meaning of the determinants should be delimited. This type of test would have another advantage : it could be used with processed products because it does not require, in contrast to AFLP, a good quality DNA.**

Moreover, AFLP allows virtually infinite combinations (restriction enzyme pairs, selective nucleotides). **The AFLP technique**, which our laboratory is proficient in, **can further be used for research into new discriminant bands between fast-growing and slow-growing chicken strains. Characterisation of the slow-growing strains most commonly used commercially could be envisaged in this framework. Commercial exploitation of the research results (patents, analysis kits) is not unrealistic.**

With regard to animal feed, determining the origin of particles by means of near infrared microscopy appears to be the most promising issue for rapid detection of animal proteins and fats in feed. The application of the technique is developed at the Department QUALITY OF AGRICULTURAL PRODUCTS at the GEMBLOUX AGRICULTURAL RESEARCH CENTRE. It requires to create libraries of raw material spectra which include the variety of ingredients that go into animal feed formulations.

At present, the presence of inhibitors (antibiotics or other substances) in animal feed is verified by identifying an inhibiting power with respect to various bacterial strains. Analysis of different raw materials has revealed the inhibiting action of a number of components, in particular the different ones produced by grinding cereals (bran, middlings, wheat feed). Other matrices (lucerne meal), after aqueous extraction, reveal an area of inhibition. Bacterial strains have been isolated from samples of raw materials showing a positive result. One of these, *Bacillus licheniformis*, which was active against the test strains, is responsible for the production of a molecule used in animal feed: bacitracin. From the chromatographic tests carried out it is evident that this antibiotic is in no way responsible for the positive results. Similar results have been observed with chicken meat samples producing abnormal positive results: a strain of *Klebsiella oxytoca* was isolated from the meat, this bacterium also show a bactericidal effect on the test strains used.

An HPLC method coupled with a fluorescence detector has been developed in order to reveal coccidiostats in feed (sulphamides). The extraction developed and the HPLC parameters confirmed the purity of the peaks obtained.

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NITRATE STANDARDS AND INTEGRATED NITROGEN MANAGEMENT IN ROW CROPS

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SUMMARY

In the frame of sustainable agriculture and environmental normalisation, the Crop Production Department of CRA Gembloux has developed a research focused on the risks of enrichment of groundwater through nitrate coming from agricultural activities. Indeed, it clearly appears that an excessive nitrogen fertilisation always leads, in addition to higher costs, to high nitrogen residues in the soil at harvest, also increased by mineralisation process of crop residues in autumn, among other sources.

Our approach is to propound a tool for the management of nitrogen fertilisation of main crops in Belgium. We have choice the software AZOBIL (INRA, Laon France) studied since 1994 in our Department essentially in cereals, sugar beet and potato crops. To validate AZOBIL we have considered the crop yield in term of quantity and quality (no yield decrease due to nitrogen management) and mineral nitrogen residues in the soil at harvest.

Field trials were conducted on main crops in Belgium, for which nitrate leaching risks are well known and increase from winter wheat to sugar beet to silage maize to potato and to vegetable crops. Results shows that the use of AZOBIL does not lead to yield decrease, whatever the crop. Moreover, in most of the situations, we have measured, for AZOBIL treatment, a diminution of soil mineral nitrogen residues at harvest. In some cases, it corresponds to less nitrogen in the soil profile and in other cases, crop residues are less abundant and then less pollutant in term of enrichment of soil profile by potentially leachable nitrate in depth.

To conclude this study, the following recommendations can be propound. Although its complexity, the nitrogen fertilisation management can be improved by a software like AZOBIL. Its use is beneficial for the environment since it leads always to a reduction of the nitrogen rate usually applied by farmers and since it does not penalize the user. Moreover, AZOBIL is easy to run and can be adapted in function of local conditions, which still allow to precise the nitrogen advice. Nevertheless the management of some elements remains difficult, like the consideration of the real contributions of manures in term of mineral nitrogen during the crop period. The close relation between mineralisation and temperature give rise to uncertainties but the regular analyses of manure to determine their nitrogen concentrations can help to give a more accurate advice. A lack of precision is also monitored when AZOBIL is used for field vegetable crops. AZOBIL were initially designed for arable main crops. More information will be soon given from INRA of Laon for vegetables. Moreover, our

Department begin a new research project on this problematic. Particular attention must also be paid to the high sensitivity of vegetable crops to climatic conditions.

During this work, we have demonstrated the usefulness of the management of nitrogen fertilisation at the rotation scale. Situations for which a great excess of nitrogen in the soil is monitored can not be corrected after one year but it is necessary to consider the whole crops rotation.

The actual Walloon legislation concerning mineral nitrogen residues in the soil is not sufficiently detailed. Indeed, the Ministerial law of the Walloon Government of the 14th of March 1995 determines at 50 kg N/ha the quantity on mineral nitrogen acceptable on the first 60 centimetres of the soil profile. Our researches shown that adaptations must be realised taken into account not only the crop species (f.i. spinach is different from sugar beet) but also the pattern of the soil profile (excess in the top or in the lower part of the profile) and the rotation (estimate the potential of the following crop to absorb nitrogen).

In this way, the recent propositions of the Walloon Region to the European Commission in the frame of the application of the Nitrate Directive are a novelty in terms of potentially leachable nitrogen (PLN) in the soil profile. A maximum PLN (taxation level) would be annually defined in function of information on weather conditions, crops and soil texture collected in basic representative situations.

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TRACING AND AUTHENTICATION OF FOOD/FEEDSTUFFS BASED ON GENETICALLY-MODIFIED ORGANISMS

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DESCRIPTION

This is the description of the project as it has been published on Fedra, part of the website http://www.belspo.be (no executive summary received).

The present project aims at the assessment of a tracing and authentication methodology of materials or products based on - or derived from genetically-modified organisms (GMO).

In 96 and 97, consents were granted on basis of directive 90/220/EEC for the placing on the EU market of grains from transgenic plants as raw material to be processed in the food or feed pathways; glyphosate-tolerant sojabeans (decision 96/281/EC, JO L107/10 of 30.04.96) and insect-resistant, glufosinate-tolerant maize (decision 97/98/EC, JO L31/69 of 1.02.97).

As a result, the EU and particularly Belgium as exportation target of these GMO's were facing strong information requests from both the consumers, the processing and distribution sectors: the identity of the origin of consumption goods remains a major concern of the public and the authorities. The public acceptance of GMO-based product should urgently be reinforced in Belgium as abroad, because of the irreversible spreading of GMO-based materials and products on the world market.

One element -among others- helping to change public perception would be the availability of a tracing and authentication monitoring of GMO-based food products. Such methodology would both facilitate the implementation of directive 90.220/EEC or the recent Novel food EC reglement 258/97 of January 27th, 1997 by the Belgian competent authorities and rationalise the labelling of GMO-based products.

Network and Theme: Each in their own area, the 3 proposing institutes are exclusively linked to the concerned competent authorities, owns the appropriate scientific information and techniques and have legal access to legal information, the raw material, the processed material and any distributed products. The present project co-ordinates these means of the 3 institutes and focuses a common objective: the development and validation of standardised tracing and authentication methods in the case of three types of transgenic plants and derived products: sojabeans, maize and oilseed rape.

Tracing transgenes: the presence of authorised transgenes inserted in the genome of these three traditional crops provides for the first time a plant DNA tracing tool along industrial processing from the initial raw material down to the distributed products.

Authentication: the transgenes authorised by the above-mentioned decisions are well known by the co-ordinating institute. Other known transgenes are those related to the officially-authorised deliberate releases occurring in the EU on basis of the application of directive 90/220/EEC or in the world as documented by the OECD Biotrack database. The scientific and patent literature brings further DNA sequences information's about the still-to-be-released transgenic plants under development. The co-ordinating institute currently feeds a database of the transgenes under research and development in the world. Beside the authentication of transgene, the authentication of a GMO as pure product or mixed in raw material also further requires the knowledge of the genomic fingerprinting of the genomic inserts and the knowledge of the plant species or variety. For these, the present project foresees the development of additional methods for the rapid certification of authorised GMO's and the detection of unknown GMO's in raw material. Exploitation of ribosomal RNA sequences, species-specific genes and GMO-specific AFLP patterns is planed.

Methods: the chosen methods are derived from the classical PCR-based methods. The PCR is a both highly sensitive and specific method for the isolation of DNA or RNA sequences of interest and for genome fingerprinting.

Tasks and normative frame: The first task will be to seek DNA in a variety of raw and industrially-processed materials representative of the transformation pathways. The second task will be to define the tracing limits of the methodology. The third task will be to develop and extend the authentication means to be applied to the identification of authorised GMO products, their plant variety and to the detection of potential unknown GMO's. As a methodological safety, a bank of DNA will be prepared from non-genetically modified crops to be used as negative reference material. These task have to be carried out with reference to CEN TC233 prenorms, under GLP and on basis of the advises of consumers and distribution groups.

Expected output: validated public-domain PCR primers, science-based interpretation of "substantial equivalence" of Novel foods, certification of transgenic origins, normative data for product labelling, a monitoring expertise applicable to R&D and an overall increased safety perception of the public and the industry.

NP/67/27

A RAPID IN VITRO BIOASSAY TO CONTROL LEVELS OF DIOXIN-LIKE SUBSTANCES IN FOOD SAMPLES

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EXECUTIVE SUMMARY

Healthy products and food safety is what consumers expect from agriculture and the food industry. Adequate controls to protect public health, based on scientific knowledge, are a major task for the authorities.

This report describes the results of a three years study, performed at Vito (the Flemish Institute of Technological Research), which aimed to develop a monitoring tool to detect elevated concentrations of persistent polyhalogenated aromatic hydrocarbons (PHAHs) with dioxin-like activity in food products. This project was initiated well before the dioxin crisis. The Belgian dioxin crisis was a sinister demonstration of the urgent need for adequate food monotoring programs for dioxins and related compounds in human food products. The benefits of an adequate control program for society, for economy and for public health has been widely accepted now. It fits into the framework of sustainable development.

As a result of this study we have now available a validated bioassay which is ready for use to screen different food items for their concentration of compounds with dioxin-like activity. After comparing various bioassays we chose for the CALUX assay, based on genetically engineered rat liver cells cultured *in vitro*. The cells emit a light signal that can be quantified if dioxin-like substances bind to the intracellular arylhydrocarbon (Ah) receptor. The binding affinity to the receptor has been shown directly related with toxic potency. The most toxic dioxin congener (tetrachloro-p-dioxin = 2,3,7,8 TCDD) has the highest known binding affinity and is used as a standard in each series of measurements. The results of the bioassay measurements are expressed in toxic equivalents of 2,3,7,8-TCDD (TEQ). The cellular method was optimised and miniaturised to allow routine-analyses in 96- well plates. A further step was to expose the cells to PHAHs, which are present in milkproducts, in eggs, and meat. A standard procedure was developed for fat extraction and further isolation of the compounds from the fat extract. This step was necessary to overcome possible interference of the light signal with compounds of the food matrix. A major challenge was to obtain low background signals and a low limit of detection, this was technically more difficult than initially expected. Starting from 1 g of animal fat, we obtained a limit of detection of about 0,1 pg TEQ/ g lipid and a limit of quantification of about 0,4 pg TEQ/ g lipid which is comparable to the results obtained with chemical analysis of dioxins/ furans with HRGC-MS. The repeatability (variability of samples analysed in the same run) showed a coefficient of variation (CV) of 10%, intralaboratory reproducibility based on independent runs of the same samples showed more variation (CV of 26% for samples above 2 pgTEQ/ g lipid).

Results from the bioassay have been compared to results from chemical analyses (HRGC-MS) on the same food substances (62 milksamples, 17 cheese samples, 6 samples of animal fat, 5 samples of eggs). In milk samples, a significant correlation ($p<0,0001$) was found between CALUX-TEQ and TEQ-dioxins/furans, TEQ- PCBs and TEQ (PCDD/F + PCBs) with respective Spearman 's Rank correlation coefficients of 0,72; 0,67; 0,73. Results from eggs were also significantly correlated. No significant correlation was observed with the cheese data and the meat fat data. This may be due to the low concentrations measured in the cheese samples (average below 1 pg TEQ/ g lipid) and the few meat samples that were analysed within a narrow concentration range. All samples which showed chemical TEQ values above the current limit values in Belgium showed elevated CALUX TEQ concentrations, above 6 pg TEQ adjusted on a lipid weight basis. No false negative results were obtained.

The CALUX- TEQ values were higher than the chemically determined PCDD/F-TEQ or the PCB-TEQ. However if the TEQ values of PCDD/F and PCBs were combined, the CALUX TEQ was somewhat lower. The bioassay measures directly the dioxin activity of the mixture of compounds present in the extracts, taking into account dioxins, furans and coplanar PCBs and their possible interactions. This may be toxicologically more relevant than the information obtained from chemical measurements. It certainly guarantees a more conservative approach than if only dioxins/furans TEQ values are determined as is usually done.

Recommendations

1. *Based on the good correlation between CALUX -TEQ and chemically measured TEQ levels the CALUX bioassay can be recommended as a screening tool for routine measurement of potentially toxic PHAHs in food. The bioassay allows to screen rapidly a large number of food samples. Positive samples are identified. The bioassay is however not specific. In order to identify the compounds that contribute to an elevated CALUX signal, subsequent chemical analyses are needed to identify the nature of the compounds that contribute to the toxic signal. We suggest a protocol in which chemical analyses are limited to those food items that turn out positive in the bioassay. These costly and laborious chemical analyses should be applied on positive samples, which are definitely the relevant ones for further research. Since no false negatives are identified with the bioassay in our study, this approach can be highly recommended as a result of our study.*

2. *If CALUX TEQ results are increased in comparison with the results from chemical analyses (marker PCBs or PCDD/F- TEQ), the samples may need*

further follow up to identify the chemicals which contribute to the CALUX signal. It may be possible to identify contamination with other persistent halogenated hydrocarbons that are not included in the conventional package of chemical measurements.

In a second part of the study, various meat samples (34 samples) and fishery products (34 samples) were purchased from different food stores and processed for CALUX measurements. The study was too limited to obtain representative data for the selected food items and to draw definite conclusions. Meat samples contained average levels of CALUX- TEQ between 1 and 2 pg TEQ/g lipid, fish samples showed concentrations that went up to 39 pg TEQ/g lipid. If expressed per g fresh tissue, median concentrations were highest in herring (2,2 pg TEQ), followed by pork (0,7 pg TEQ) and eggs (0,7 pg TEQ). For risk assessment (contribution to TEQ body burden) we need to take also into account the relative contribution of these food items to the average diet. Using published food consumption data from abroad (since no up to date food consumption data exist in Belgium), combined with the median TEQ concentrations measured in this study, daily TEQ intake levels were calculated. Due to the above mentioned limitations they should be considered only as indicative.

Apart from the absolute TEQ values, interesting information can be obtained from the variability among samples from a selected foodproduct.

Pooled samples (milk, eggs) showed relative low coefficients of variation, while CV of individual milk and chicken samples went up to 0,80 and 0,50 respectively. Beef and pork samples showed less variation. Fish samples also, had high variation coefficients, except for the salmon samples.

The bioassay allows to chararcterise a group of food items based on their TEQ concentration and on the heterogeneity among samples in TEQ values. This information allows prioritisation of food items that need further follow up.

Recommendations

1. *This assay can be used to detect rapidly, unknown contamination in food samples and may help to identify yet unknown sources of contamination. We recommend to use this screening assay regularly on a representative set of samples. Samples should be analysed on an individual basis. Samples with high coefficients of variation indicate that part of the consumers may be exposed to elevated TEQ concentrations in their food which do not occur in food items from a different origin. Food items showing high coefficients of variation require further systematic screening in relation to their origin or their processing.*

2. *CALUX is a promising tool for monitoring as part of a food surveillance campaign or for study of environmental levels of PHAHs in animal fat tissue. The next step for further implementation is an international validation study which is a prerequisite for further acceptance.*

NP/D1/28

METHOD FOR MEASURING SCUFFING ON RETURNABLE GLASS CONTAINERS

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1. ABSTRACT

According to the European wish (directive 94/62/CE) to encourage the reuse of glass containers, an ever increasing percentage of glass bottles are returnable on the North European market. In other words, the bottler takes increasing responsibility for bottles after use. The bottler therefore deals with the washing of bottles, filling them, labelling them and storing them. The bottles are then put back into the distribution circuit for a new cycle of use.

Since the glass which forms the bottles is a brittle material, its potentially very high mechanical resistance is weakened by the presence of defects, which seldom occur in the mass of the glass but are frequently found on its surface. It is therefore of prime importance to protect or strengthen the surface of the glass with one or more films composed of metal oxides or organic molecules. Nevertheless, despite the presence of such treatments, the surface of bottles becomes scuffed after multiple use. As a result of bottle handling and washing operations, whitish surface marks appear. They are mainly present at the different friction points of bottles when they knock against each other, on conveyor belts for example. The term “scuffing” is given to those marks in the jargon of glass-makers and bottlers. They alter not only the mechanical resistance of the glass but especially its transparency and its visual attractiveness.

This article aims at developing a measuring method and apparatus to quantify scuffing. The interest of such a method is based on the following advantages:

- the possibility of defining a tolerable scuffing threshold,
- having a means to determine the rate of scuffing generated by a bottling line,
- being able to adjust and control the effectiveness of new means aimed at fighting the phenomenon of scuffing.

In order to approach this study, a prior analysis concerning the problem and the source of scuffing was made on the basis of bibliographical information. Next, a critical examination of the methods potentially usable to quantify scuffing led to the selection of a measuring principle used for the design of the prototype. First experiments were carried out to validate the apparatus.

2. METHOD CHOSEN AND DEVELOPMENT OF THE APPARATUS

It is admitted by experts on the subject that the generation of scuffing is caused by the combination of damage of a chemical and mechanical kind arising on the surface of the glass. Scuffing is not a physical scale of size. Outward signs characterising it therefore have to be identified and the means found to measure those signs. Several techniques may be considered to quantify a deterioration of the surface condition of glass such as scuffing: visual evaluation, measurement of the loss of weight, of roughness, transmission or diffuse reflection. All these methods have advantages and disadvantages which have been examined. It seemed to us that diffuse reflection method best met the constraints of the apparatus to be designed; in other words, it enables a direct, accurate and fast measurement of scuffing regardless of the influence of external parameters.

The basic principle of the instrument developed by the InV, the “scuffmeter”, consists of sending a source of light onto the side wall of the bottle using an emitter-sensor cell. The direction of the ray of light is different from the normal so as to be able to differentiate normal reflection and reflection due to the surface defects of the analysed bottle. The information received on the cell is sent to a programmable automatic device and processed by software programs to build up the cartography of the surface condition of bottles. That information is converted into rates of scuffing, which correspond to the ratio of the number of positive responses (= number of defects or scuffs) over the total number of measurements taken.

The selected sensor is an inexpensive, single, digital, photoelectric sensor operating in the “all or nothing” mode in relation to an adjustable threshold of light reflected by diffusion. The sensor is included in a cell where the emitter is also located (light beam of a constant diameter). The cell driven by a stepping motor moves along a vertical axis. The bottle is held through an AGR (American Glass Research, Inc.) type grapping device, that can be manually adjusted and an automatic device operates its rotation. The measurement is taken initially at the bottom of the bottle over the whole circumference and the cell rises by one vertical step after each revolution. Binary type data (defect or no defect) are captured as far as the shoulder of the bottle.

3. TESTS AND RESULTS

First tests allowed to select the best position for the sensor (distance between sensor and bottle is 22 mm, angle between normal and bottle is 17°), given the geometry of the bottle.

3.1 Validation

We measured the scuffing rate of 10 bottles taken on a washing line to validate our method. These bottles have been first measured by TNO. The rate of scuffing of those bottles decreases approximately in a linear way as a function of wearing rate and are well correlated with TNO measurements (table 1).

Moreover, when measuring scuffing only on the two most worn rings (upper and bottom zones) instead of on the whole body of the bottle, the relation has the same behavior as that obtained just before. It is then possible, if necessary, to optimize the measurement time of scuffing. Indeed, the measurement of the two rings only could accelerate the measuring time which is not negligeable when considering a use of the method for on-line measurements.

Table 1 : comparison between measurements done by TNO and those obtained with our apparatus.

Standard	1	2	3	4	5	6	7	8	9	10
mV (TNO)	1000	900	800	700	600	500	400	300	200	100
intensity	heavy				average					light
rate	0.399	0.322	0.318	0.298	0.209	0.131	0.126	0.069	0.019	0.042
scuffs	3192	2577	2540	2380	1669	1047	1006	555	157	334

On another hand, two series of samples that have been submitted to 5, 10, 15, and 20 wearing cycles have been analyzed. Even if the number of samples is limited, they can validate the technical choices we made for the prototype:

- a clear distinction can be made between the wearing obtained after several cycles,
- the dispersion of results is small (low standard deviation),
- the scuffing rate becomes very important after 10 wearing cycles (similar results as for visual observation),
- the noise level, characteristic of the presence of other surface defects (no wearing cycle), is rather low,

- the maximum error obtained on a same bottle is lower than 5%, which is quite compatible with a good reproductibility.

3.2 Relationship between rate of scuffing and mechanical resistance

Bottlers wish to know the relationship that exists between the rate of scuffing of returnable bottles and their mechanical resistance. Therefore, a set of worn bottles of the same model has been used.

The rate of scuffing is measured before the bottles are submitted to a destructive test of resistance to internal pressure. For comparison, new bottles resisted an internal pressure of 40 psi (pound per square inch), according to our test. When bottles are simply knocked against each other, their mechanical resistance remains the same. When bottles are scratched, the resistance to internal pressure is highly lowered and reaches values between 26 and 37 psi. When they have been submitted to several wearing cycles, their resistance can be as low as 20 psi.

These tests show that the surface treatments of returnable bottles are very good protecting agents of the glass surface. One scratch eliminates the surface treatment and lowers the resistance of the bottle which can cause very important damages to the bottling line. Such a scratch is the starting point of the scuffing phenomenon because it allows the washing solutions to reach the unprotected glass surface.

3.3 Constitution of two standard scuffed bottle series

Two series of 10 standard bottles have been constituted, on one hand to calibrate the prototype, and on the other hand to determine, together with bottlers, the rate of scuffing that can be aesthetically accepted by the client. Two types of bottles commonly used in Belgium (brown APO 25 cl and uncolored Spadel 20 cl bottles) have been scuffed during wearing cycles. This way, a standard curve is obtained, that allows to define an acceptable scuffing rate. Such a standard curve can be reproduced easily for any kind of bottle.

4. CONCLUSIONS AND PERSPECTIVES

The scuffmeter designed and used at the InV at the present time is a laboratory prototype that still requires some technical improvements to meet the requirements of production line use. Future research will concern, among others, the reduction of measuring time and the programming of automatic statistical processing of results.

NP/B3/29
NP/02/30
NP/10/31
NP/10/32

MULTI-CRITERIA AUDIT TOOL FOR THE TECHNICAL QUALITY MANAGEMENT WITHIN THE AGRI-FOOD SME'S

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EXECUTIVE SUMMARY

The Centre for Education and Research for Food Industries (CERIA), the State University of Ghent (RUG - Faculty of Agriculture & Applied Biological Sciences) and the Catholic University of Louvain-la-Neuve (UCL - Faculty of Law (Center of Consumerrights) and the Faculty of Econ. Soc. & Pol. Sciences (Department of Communication)) collaborated in this research project.

The project started early April '98 and was finalised by the end of May '00. The most prominent goal of the study was to set up a multi-criteria evaluation document for agri-food SME's concerning the conformity of the internal quality control with the regional legislation requirements (Flemish, Brussels and Walloon).

Within the framework of the study an assessment was made to develop a tool that can be used by all the SME managers from the different food sectors. An SME is identified as an enterprise that employs *less than fifty persons.*

The document comprises three parts: the first is related to the lay-out of the plants, the manufacture and storage processes with particular interest in cleaning and desinfection, waste management and the optimal use of raw materials, water and energy. The second part investigates the SME's with regard to the rights and expectations of consumers in three essential points: information by means of product description, safety and complaint handling. The third part deals with internal and external communication of the SME's: guaranteeing the transfer of internal information between functions, continuous training of the personnel and the quality of clients relations.

The questionnaire was developed and tested in close collaboration with 22 SME's spread over the three Belgian regions. If necessary, the questions were adjusted in relation to their pertinence and understanding. The results have demonstrated that the managers concerned have been able to identify the weak and key points of their enterprises.

This integrated audit instrument answers the demands of legislation requirements (Responsibility of the Producer). It also stimulates the willingness of the SME's towards quality assurance and will enable the completing of the national database on quality policy in agri-food production.

In the meantime, the document has been submitted to a Belgian publisher - La Charte - Editions Juridiques, rue Guimard, 19 - 1040 Brussels for publication of two

editions: a French edition for the SME's from the Walloon and Brussels regions and a Flemish edition for the SME's from the Flemish region.

Each edition contains an audit questionnaire subdivided into three parts and included marks related to the conformity of the prevailing legislation and good manufacturing practice.

NP/43/33
NP/01/34

INTRINSIC INDICATORS FOR PROCESSED MILK AUTHENTICITY

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1. ACTIVITIES AND PROGRESSES

The following activities were carried out:

- An inventarisation of the current situation in the Belgian consumption milk industry is made by the organisation of inquiries.
- A detailed kinetic study is executed on intrinsic indicators of milk as a basis for the development of indicators for processing authenticity and the development of a mathematical model.
- A mathematical model based on kinetical data, that allows formulating the process conditions, which cause the desired microbial inactivation with minimal fouling of the heat exchangers, is developed for different types of the heat treatment systems.
- Methods for the determination of potential intrinsic indicators are optimised and developed.

2. RESULTS

The following results were obtained:

- The organisation of 2 inquiries among the most important Belgian consumption milk producing plants provided an inventory of the current situation in the Belgian dairy industry.
- Improved methods were developed for the determination of lactoperoxidase, hydroxymethylfurfural, and alkaline phosphatase (quantitative).
- Mathematical models for different heat treatment systems were formulated.
- The kinetics of physico-chemical changes of milk as a basis for processed milk authenticity indicators or relevant for fouling of heat exchangers were experimentally determined.

NP/12/35
NP/02/36
NP/B4/37

INTEGRATED STRATEGY FOR THE QUALITATIVE AND QUANTITATIVE ANALYSIS OF RESIDUES OF ANTIMICROBIAL SUBSTANCES IN FOODPRODUCTS

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1. EXECUTIVE SUMMARY

In the framework of the "Scientific Support Plan for a Sustainable Development Policy in the field of Standards for Food Products", we have developed two actions :

1.1 Standardisation of hormone and veterinary drug residue analysis in animal products

(March 1997 – February 1998))

A new database was created which provides a carefully judged inventory of analytical methods available for the determination of residues of growth promoters (steroidal anabolic hormones, ß-agonists and glucocorticoids) and veterinary drugs (antibiotics and growth inhibitors), which are or will be regulated by the European Union legal acts.

Other parts of the database involve informations on:

- legislation (Belgian and European),
- toxicological aspects of residues,
- statistics of controls performed by the authorities (Ministry of Agriculture and Institute of Veterinary Expertise – IEV – of the Ministry of Public Health).

This database is available on the Internet at: *http://139.165.180.63/OSTC/*

1.2 Integrated strategy for the qualitative and quantitative analysis of residues of antimicrobial substances in food products

(from March 1998 to February 2001)

The project aimed at demonstrating the feasibility of such an integrated strategy. The strategy is based on the availability of appropriate immunoassays or other biochemical tests and of suitable chemical methodology which is considered commonly available to a well equipped control laboratory.

A pilot methodology for the identification, the confirmation and the quantitative determination of residues of commonly encountered antibacterial substances in animal tissue and products is set up. After thorough validation in agreement with the internationally accepted standards, the methodology have been applied in practice on real samples collected in slaughterhouses by IEV meat inspectors. These

samples had been considered as positive for the presence of antibacterial agents after testing with the “New Belgian Kidney Test”. We received also from IEV kidney and meat samples from animals recognized as negative for the presence of antibacterial substances in their kidney according to the official control. These samples were used as “blanks” to set up our methods of analysis and during their validation in our quality controls.

Presently in Belgium, the test which is applied to determine if a food sample is positive or negative for the presence of antibacterial substances, is the “Belgian Kidney test”. This “pre-screening” microbiological test, which is applied on kidneys of slaughtered animals, is based on the antibacterial activity of antibiotics. It only allows to detect the presence of inhibiting substances in kidney exsudate. But it does not allow the identification of the inhibiting substances nor the quantification of their concentrations in edible parts of the animal carcass, particularly in skeletal muscles (meat). To make progress in the application of MRLs, it is thus necessary to apply simple and rapid methods which could allow the identification of antimicrobial substances. These “new” methods will thus complete the results obtained from the kidney test in order to establish if the antibacterial substance is permitted or banned in animal products and to determine if its concentration is compatible with the MRL regulation.

An efficient system for the control of antibacterial residues should consist of four stages:

1. Pre-screening at the level of the slaughterhouses by means of a microbiological test for the presence of bacterial growth inhibiting substances. This phase is already fully operational in Belgium (Belgian kidney test).

2. Selective screening of the positives, obtained sub 1, by means of immunoassays in order to come to an identification of the group of growth inhibitors (sulphonamides, beta-lactams, tetracyclins, aminoglycosides, macrolides, (fluoro)quinolones, phenicols)

3. Chemical identification (using GC, GC/MS, HPLC/UV, LC/MS methods) within the group of the individual growth inhibitors.

4. Quantitative assay of the identified residue in view of the established Maximum Residue Limit (Council Regulation N° 2377/90).

The latter three phases are not yet operational due to the lack of an adequate analytical strategy.

The project aims at demonstrating the feasibility of such an integrated strategy. After a thorough literature search and owing to our developing expertise in the framework of *part 1* of the project, it became clear that such a system does not yet exist. The selection of the antibacterials which have to be analysed was based on the previous experience of both laboratories involved in this project in the determination of given families of antibacterial agents and the availability of appropriate immunoassays, or other biochemical tests, and of suitable physicochemical methodology, which is commonly available to a well equipped laboratory.

The antibacterials which were most often found as residues in animal products belong to one of the following seven families of substances: beta-lactams, (fluoro)quinolones, macrolides, chloramphenicol, tetracyclines, aminoglycosides and sulphonamides.

Previous experience of the services of the Ministry of Public Health in the control of residues in animal products has shown that sulphonamides, tetracyclines and beta-lactam antibiotics are the most often detected antibacterials in kidney and meat. For this reason, only samples which were found positives in the kidney test but negative for these 3 families, have been analysed for the presence of chloramphenicol, aminoglycosides and macrolides.

The objectives of the two projects were the following:

2. RESULTS

2.1 University of Ghent work package

The antibacterial families which were studied in Ghent involved: sulphonamides, tetracyclines and aminoglycosides.

Sulphonamides

Screening

As no multiresidue ELISA kit was available on the market for sulphonamides, High Performance Thin Layer Chromatography (HPTLC) was selected as screening method. After development in HPTLC, the spots corresponding to the residues were still visible at concentrations lower than the MRLs (100 µg/kg). The extraction yield was very high and no interferences were noted. This technique allows the determination of 30 samples per day and per analyst. A total of 161 samples (kidney

+ muscle) were analysed with this method, from which 21 (13%) were found positive for the presence of sulphonamides.

Confirmation by LC-MS/MS

A simple extraction procedure was adopted. After optimalization of the chromatography as well as the mass spectrometry conditions, the method was validate taking into account the following parameters: linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) for kidney as well as for meat samples. It appeared that that this method is in agreement with the quality criteria defined in the European Union for analysing residues in food.

From the 42 samples (kidney and muscle) found positive in the screening phase, 28 (67%) were confirmed by GC-MS/MS without ambiguity. This corresponds to about 1/3 of the samples considered as positive after the screening but which were found in reality false positive when MRL values are taken in consideration.

Tetracyclines

Screening

The applicability of the commercial Ridascreen tetracycline kit (R-Biopharm) was tested. A total of 104 meat and 99 kidney samples, which had been found positive in the Belgian Kidney test, were analysed. For the meat samples, 28 (27%) appeared to contain more than 100ppb tetracyclines (MRL value). For the kidney samples, 13 were considered as positive at this stage. This represents a total of 41 positive samples.

Confirmation by LC-MS/MS

A simple extraction procedure was adopted. The analyses were performed on the same apparatus as used for sulphonamides. After optimalization , the method was validated using the same criteria as for sulphonamides.

From the 41 samples (kidney and muscle) found positive in the screening phase, 20 were confirmed by GC-MS/MS without ambiguity. This corresponds to about half of the samples considered as positive after the screening but which were found in reality false positive when MRL values are taken in consideration.

Aminoglycosides

Screening

Neomycin was selected as representative of this large group of antibiotics. Actually, it is a mixture of 3 different forms: neomycine A,B and C. Several assay kits are available. The EIA kit from Euro-Diagnostica was finally selected after a short comparison study.

Large variations were observed in the results and it was not possible to overcome this problem within the time allowed by this research. The difficulties are probably due to unreproducibility of the extraction yields.

From the remaining samples that had not been found positives for sulphonamides nor tetracyclins, namely 65 kidneys and 66 meat samples, 7 (11%) kidneys and 5 (8%) meat samples were tested as positive in screening. Due to the lack of precision of the results, it was not possible to compare to the neomycin MRL value.

Confirmation by LC-MS/MS

Due to the difficulties encountered with the yield of extraction, it was considered that the otpimization of the LC-MS/MS method of analysis was not possible at this stage.

2.2 University of Liège work package

The antibacterial families which were studied in Liège involved: beta-lactam antibiotics, macrolides and chloramphenicol. During the course of this contract, (fluoro)quinolones were also considered taking into account their particular importance in the problem of the increase of antibacterial resistance to antibiotics.

Beta-lactam antibiotics

Screening

We have adapted, validated and use, for analysing this type of residues in kidney and meat, a new test on strip, Beta-STAR (UCB Bioproducts), marketed for the antibiotic monitoring in milk. For animal tissue analysis, the strip test was preceded with a solid phase extraction (hydroxyapatite columns) of contaminating proteins in the aqueous extract. In these conditions, penicilline G, ampicilline and amoxicilline are detected at concentrations close to their MRLs. For oxacilline and cloxacilline, they are detected concentrations lower than LMR/2.

Confirmation / quantification

High performance liquid chromatography (HPLC) was selected as the analytical method for confirming the presence of beta-lactams in kidney or meat samples and for their quantitation. It was observed that it is mandatory to adopt special working

conditions to avoid degradation of these substances during the analytical process. LOD was evaluated to 15 and 75 µg/kg according to the type of compound. LOQ is 25 µg/kg for penicilline G, amoxicilline and ampicilline and 150 µg/kg for oxacilline and cloxacilline. This method of analysis is thus well suited for penicilline G, amoxicilline and ampicilline (MRL = 50 µg/kg) and for oxacilline and cloxacilline (MRL = 300 µg/kg).

From the remaining samples that had not been found positives for other antibacterials, namely 17 kidney samples examined, only one was positive in screening and confirmed for ampicilline at 1553 µg/kg.

Macrolides

Screening

The method used for the detection of macrolide residues was a radio-receptor assay developed and validated in our laboratory. This technique was applied for analysing kidney and meat samples collected by IEV meat inspectors.

Confirmation / quantification

A LC-MS/MS method was developed and partly validated.

From the 17 kidney samples examined, only one was positive in screening and the presence of spiramycine was confirmed at 93.5 µg/kg.

Chloramphenicol

Screening

The ELISA Ridascreen was used for the determination of chloramphenicol (a banned substance in veterinary medicine). Other antibacterial compounds of this family, such as thiamphenicol, were not taken in consideration due to the lack of multiresidue test kits available on the market.

Confirmation / quantification

A LC-MS/MS method was developed for the quantitative analysis of chloramphenicol.

From the 17 kidney samples examined, 4 were positive positive and their concentrations were estimated to 0.4, 0.6 (2) and 1.2 µg/kg.

(Fluoro)quinolones

Screening

Screening test is not commercially available. A new biochemical test is still in development at the University of Liège.

For this reason, the samples collected by IEV were not examined for the presence of this type or residues.

Confirmation / quantification

The conditions of extraction and purification of these residues from kidney and meat samples have been studied for the main substances of this family of antibacterials and a LC-MS/MS method of analysis was set up and validated. It was found applicable as a multiresidue method. Quantitation will soon be optimised by using a new deuterated standard, this will allow a complete validation of the method.

Our project had to lead to:

1) a strengthening and an improvement of the link between the scientific potential of research centres (Universities of Ghent and Liège) and the regulatory authorities concerned by the standardisation in the food product sector (within the future Federal Agency for the Safety of the Food Chain) and also with the OIVO/CRIOC (a Belgian Centre of Research and Information of Consumer Organizations). The OIVO/CRIOC will be informed about the results of the project and coached in the translation towards the consumers.

2) an acceleration and a better coordination of the standardization process in a context of sustainable development in the sector of animal productions.

3. CONCLUSIONS AND RECOMMANDATIONS

The important changes in the strategy of control of residues in foodstuffs and in food products required in the European Directives (EC/96/23) and Council Regulation EEC N°2377/90 that have been implemented rather recently require also a modification of the strategy of the use in the laboratories of analytical methods for the determination of veterinary drug residues and particularly of antibiotics. For these last compounds, the question is asked about the demonstration of the presence of substances inhibiting bacterial growth that could no longer be sufficient to reject the

animal carcass: after identification of the inhibiting substance, its concentration in animal products edible for human consumers would have to be determined and compared to the Maximum Residue Limits (MRLs) in the corresponding foodstuffs. Reliable and reproducible quantitative methods of analysis are thus needed. This will be possible only after complete validation and standardisation of analytical methods.

Our project has largely reached its objectives:

- an inventory of the control tools consisting of commercially available methods for the post-screening of antibiotic residues was established. Some of them have been applied to the determination of antibiotic residues in animal kidneys. Their complete validation and standardisation are in progress;

- published physico-chemical methods for the confirmatory analysis and quantitation of antibiotic residues have been applied to kidney samples collected in Belgian slaughterhouses. Those which were found satisfactory were partly validated and are on the verge to be standardised in view of their inter-laboratory evaluation;

- new analytical approaches were followed when needed after a negative evaluation of the performances of existing methods of analysis;

- progress have been done is the development of laboratory networks at the national and European levels in the field of the control of antibiotic residues in foodstuffs of animal origin.

Our project was clearly in line with the general objectives of the *Scientific Support Plan for a Sustainable Development Policy* in the food product sector for the harmonization of methods of analysis. This should allow a better protection of the consumer health concerning potentially harmful residues of certain veterinary drugs such as antibiotics.

The validation of the analytical methods developed and tested in our project will have still to be done in close collaboration with other laboratories involved in the official control of veterinary drug residues in foodstuffs of animal origin. This will contribute to the integration of results and will allow identification of domains in the food production chain for which an effort of standardization should be needed in the frame work of sustainable development. The project will contribute to the creation of an inventory of European and worldwide initiatives and to the development of reliable data bank largely available. It will examine actions taken at the Belgian and international level in the field of food product standards (more precisely, methods of residue analysis in

food products) in a context of sustainable development and it will allow the definition of the Belgian contribution at the international level (especially the European level).

Validation of analytical methods developed and tested in our project will still have to be completed in collaboration with other laboratories involved in the official control fo residues. This will allow the identification of domains in the sector of the chain of food production in which a special standardisation effort is still necessary in the framework of sustainable development. The project will have to contribute in the setting up of an inventory of the initiatives at European and worldwide levels et in the development of databank easily available. It will examine the actions conducted in Belgium and at the international level in the domain of standardisation of food products (more specifically concerning the analytical methods of residues in foodstuffs of animal origin).