SCF/CS/ NF/ LABEL/13 Rev. 4

OPINION

CONCERNING THE SCIENTIFIC BASIS FOR DETERMINING WHETHER FOOD PRODUCTS, DERIVED FROM GENETICALLY MODIFIED SOYA AND FROM GENETICALLY MODIFIED MAIZE, COULD BE INCLUDED IN A LIST OF FOOD PRODUCTS WHICH DO NOT REQUIRE LABELLING BECAUSE THEY DO NOT CONTAIN (DETECTABLE) TRACES OF DNA OR PROTEIN

(expressed on 17 June 1999)

Terms of Reference

To provide answers to questions of the Commission regarding the inclusion of highly refined oils and of certain products derived from starch in a list of products not requiring labelling with respect to any genetic modification as they do not contain detectable traces of DNA or protein resulting from such genetic modification.

1. Highly refined oil

Member States' contributions tend to suggest that highly refined oils do not contain either native or inserted DNA or protein, while cold-pressed oils would. In this respect, the Commission wishes to put to the SCF a number of questions:

1. Is DNA or protein to be found only in cold-pressed oil?
2. Are there refining processes ensuring that DNA/protein are removed efficiently from the oil?
3. Are the processes/procedures used by industry today ensuring that DNA/protein are efficiently removed?
4. Consequently, what type of oils could be included in the list?

2. Products derived from Starch

As regards starch it would appear from Member States' contributions that in maize starch as such native and inserted DNA/protein could be found. However, there are apparently several products derived from starch, which probably contain no DNA or protein. The Commission's questions in this respect are:

1. Does maize starch contain DNA or protein?
2. Which chemical or physical measures ensure that DNA and protein are removed or destroyed in a production step?
3. Is it possible to describe groups of products (e.g. products derived by hydrolysis of starch) or individual starch derived products which are free of DNA or protein?
4. Consequently, what are the products derived from starch, which could be on the "negative list"?

Background

The Committee delivered in September 1997 an opinion on the relationship between scientific data and the labelling of genetically modified foods and their derived products.[(1)]. In this opinion the Committee did not address the safety aspects of these food products because these particular aspects had already been adequately covered by provisions of Regulation 258/97/EC[(2)] and the Recommendation 97/618/EC[(3)] for the scientific evaluation of novel foods and food ingredients. The provisions of Directive 90/220/EEC[(4)] and Regulation 258/97/EC[(2)] already contain specific labelling requirements for foods and food ingredients derived from GMOs and are part of the authorisation for placing such foods or food ingredients on the market.
However, the Committee did emphasise that all novel foods accepted as being in accordance with the requirements expressed in its opinions regarding the assessment of novel foods[(5)(6)(7)] are, on the basis of current knowledge, safe for human consumption. This statement applies regardless of whether the particular novel foods must be labelled.
The Committee did also emphasise that “equivalence” is a legal term which applies to the inherent compositional characteristics of a food or food ingredient, whereas “substantial equivalence” represents safety and nutritional evaluation of such products in comparison to appropriate predecessors. It was further stressed that standardised and validated analytical methods should be employed to determine “equivalence”.

The Committee has now been requested to express an opinion on the scientific basis for determining whether food products derived from genetically modified soya and from genetically modified maize could be included in a list (referred to in Council Regulation 1139/98[(8)]) because they are processed in such a way that at least the inserted DNA and its protein expression products are removed.
The products on this list may not contain detectable traces of DNA or protein or fractions thereof, resulting from genetic modification that allow identification. They could therefore be exempted from the labelling provisions.
For this purpose the Committee took account of the conditions of use of the legal term "equivalence" of novel foods and novel food ingredients to traditional, non-GM foods and ingredients. Thus any novel food or food ingredient, for which analysis of existing data demonstrate that the characteristics assessed are the same in comparison with a conventional food or food ingredient, having regard to the accepted limits of natural variations for such characteristics, would not require specific labelling. In practice this would mean that the food or food ingredient derived from a GMO source could not be distinguished by analysis from the same product derived from a traditional source.

Since adequate processing is the basis for the removal of any genetically modified material the Committee in its opinion started from the premises that refined oils and starch hydrolysates are processed according to accepted representative production schemes.

According to information submitted to the Committee[(9)] an accepted representative industrial production process for refining crude edible oils comprises following steps. The crude oil is produced by pressing, expelling or by solvent extraction of oilseeds. The refining can be done in two ways, chemically or physically.

Chemical refining consists of degumming (mixing with phosphoric acid and water at 70-90°C and centrifugation, to remove phosphatides and proteins), neutralisation (alkaline treatment to remove free fatty acids and carbohydrates), bleaching (treatment with bleaching earth and/or filtration aids and filtration to remove pigments) and deodorization (steam injection under vacuum (2-5 mm Hg) at temperatures of 180-270°C, to remove off-flavours).
Physical refining consists of degumming (mixing of citric acid and water at various temperatures and centrifugation to remove phosphatides, proteins and carbohydrates), bleaching (treatment with bleaching earth and/or filtration acids and/or food grade acids followed by filtration to remove pigments) and deodorization (steam injection under vacuum (2-5 mm Hg) at temperatures of 180°-270° C to remove off flavours and free fatty acids).

An accepted representative production process for starch and starch hydrolysates on industrial scale comprises the following steps[(10)(11)]. Maize as raw material is first physically cleaned to remove dust and broken kernels. It is then subjected to steeping which, further on, will allow the separation of starch and proteins.
After steeping the maize is pre-ground, cycloned (removal of germs) sieved (removal of fibers) washed and centrifuged (removal of proteins) to obtain a starch slurry which is dried to produce «native» starch.
In the overall process more than 95% of proteins initially present in maize are separated.

For the production of starch hydrolysates [i.e. maltodextrins, glucose(dextrose) syrups, glucose(dextrose), isoglucose] the starch slurry is processed as follows: the slurry is first hydrolysed (acid and/or enzymatic, pH 4-4.5, temperature >105°C). In this step DNA and remaining proteins are claimed to be denatured.
The hydrolysate is further heated and filtered. The product is then processed in a stepwise manner; in a first step by adsorption on activated carbon to eliminate DNA and protein (including the amylolytic enzymes, in case of enzymatic hydrolysis); in a second step by ion exchange over anionic and cationic resins to further eliminate DNA and proteins, and possibly other remaining impurities.

1. Refined Soybean/maize Oil

Presence of DNA or protein

When crude soybean oil is centrifuged at 14000 g for 15 min (not common practice in edible oil processing) the level of genetic material (DNA) is reduced by at least a factor of 10^4 [(12)].

In the refined oil from genetically modified soybean and maize, DNA could not be detected nor PCR amplified following different DNA extraction methods[(13)]. This does not apply to Lecithin as DNA could successfully be extracted and identified by several laboratories.

In a new study presented to the Committee[(14)] procedures are described for the detection of DNA isolated from purified soy oil from commercial origin. Two types of extraction methods are described which enable the isolation of DNA.

In order to verify the quality and the quantity of the DNA preparations obtained by these methods, agarose gel electrophoresis experiments were performed. It was shown that the amount of isolated DNA was below the detection limit of the method.
Whereas in single step PCR amplification no DNA could be amplified, in nested PCR amplification experiments with specific primers, soy DNA could be visualised. These results indicate that DNA was present in the tested samples but that the absolute amounts appeared to be very low. It was also shown that the oil did not contain transgenic DNA.
On the other hand in extracts of oil derived from 100% GMO soy beans, transgenic DNA could be detected.
It was also found that the sensitivity of the test is reduced in comparison with the identical test for regular soy beans. It was therefore concluded that this limited sensitivity is most likely related to the low absolute content of DNA in the soybean oil.

In a publication on the analysis of rapeseed (*Brassica napus*) oil DNA, fragments (around 240 base pairs) were identified in both cold pressed and refined oil[(15)].

Results of analysis carried out in different laboratories confirm, that refined maize oil and soybean oil contain residual protein below 100 µg/ml.

2. Products derived from Starch

Presence of protein or DNA in maize starch

Protein

In native starch

Native starch contains a small part of the total proteins initially present in maize kernels. The typical value for total protein in starch is 0.4% (equivalent to 4.000 ppm) while the typical value for protein content in maize kernels is 9% (equivalent to 90.000 ppm). Notwithstanding the fact that more than 95% of the proteins initially present in maize kernels is separated from the starch after the steeping process, protein is still present in native starch[(10)].

In starch hydrolysates

Data show[(11)] that during the production of starch hydrolysates the protein content (measured as N x 6.25) is gradually reduced. After filtration of the hydrolysate the protein content is reduced to 1600 ppm. After further purification over activated carbon, the protein content is 800 ppm and finally after ion exchange purification the protein content is reduced to 400 ppm.
The protein content (measured as N x 6.25) in various industrial samples of starch hydrolysates (41 samples) produced in different plants from different starch manufacturers was found to be in the range of 100 to 200 ppm.

In order to assess the removal of proteins during the production process of starch hydrolysates the presence of residual proteins was analysed by HPLC coupled with UV detection. Samples were taken at an early stage of the production process (i.e. just after filtration) and at the end of the process (i.e. after purification by ion exchange). It was found that proteins with a molecular weight higher than 20.000 Dalton are already eliminated at an early stage of the process (i.e. just after

filtration and before purification on resins). It is said that the Bt-protein of (Novartis) maize is 65.000 Dalton.
Polypeptides with lower molecular weight, which might still be present after the filtration step, will most likely be eliminated during the purification on resins. The nitrogen detected in industrial starch hydrolysates (see above) probably originates from such polypeptide fragments.

In its opinion on the potential for adverse effects from the consumption of genetically modified maize[16], the Committee accepted the presence in the maize kernels of the CryIA(b) protein (Bt-protein) to be below 5 ppb and that of PAT (Phosphinothricin acetyl transferase) to be not detectable. Taking the same reduction factors as for protein (see above) the level of Bt-protein can be estimated at 0.22 ppb in starch and of the order of max 0.03 ppb in starch hydrolysates[11]. It can be expected that such a value will be below the limit of detection.
However no adequate analytical methods are presently available to test the presence of these (new) proteins or fragments thereof.

DNA

In starch and low degraded starch

Analytical data show that in industrial samples of maize starch, maize DNA is present and can be detected[10].

In purified starch hydrolysates

Testing of DNA was carried out on industrial samples of purified starch hydrolysates and on samples taken at different stages of either industrial production processes or of a pilot scale process.
Following industrially produced products were tested; maltodextrin, acid-hydrolysed glucose syrup, enzyme-hydrolysed glucose syrup, acid and enzyme hydrolysed glucose syrup, dried glucose syrup, iso-glucose, crystalline fructose, crystalline dextrose.
In a second group of experiments detection tests on DNA were carried on industrially produced maltodextrin and glucose syrup and on glucose syrup produced on pilot scale after either acid hydrolysis or enzymatic hydrolysis.

Data submitted to the Committee[10] show that out of a total of 37 different samples, analysed by five different laboratories, two samples gave unclear results. In all other samples DNA could not be detected.
The two unclear results were obtained by the same laboratory on a sample of maltodextrin and on a sample of enzyme hydrolysed glucose syrup. The laboratory indicates that for DNA analysis always duplicate copies (double classification) of the same sample are analysed. For each of the unclear analytical results, the laboratory obtained only a positive result in one of the two reactions. According to the laboratory, one reason for these unclear results could be that too little DNA could be isolated from the samples concerned.

Additional data presented to the Committee[13] carried out on a small number of samples (four) of polydextrins and maltodextrins from maize did confirm the data presented above i.e. DNA could not be detected.
All these results indicate that no DNA can be detected, in starch hydrolysates as final products or in samples taken at different stages of production.

Discussion

In the studies on refined oils from GM soybean and maize no DNA was found. On the other hand, based on the data of a new study, in soy oil from commercial origin no DNA could be amplified by single step PCR amplification but when applying nested PCR amplification with specific primers DNA could be visualised. This demonstrates the need for standardised extraction methods and standardised detection methods for DNA.

Whereas it can be presumed that accepted representative processes for oil refining could ensure the removal of a large part of protein and with a high probability also DNA the efficiency of this purification was not clearly demonstrated because of lack of standardised methods. It seems that the efficiency of purification and the elimination of the genetic material could be improved by including a step of centrifugation. Removal, identification and amplification of DNA from the oil should be considered in the light of sensitivity of analytical procedures and threshold for DNA fragment size (150 to 200 base pairs).

In the studies on starch and starch derivatives[10][11], the samples analysed were of different origin, of different size and different extraction methods for DNA were used. Indications are that the extraction efficiency for nucleic acids of the different methods presently used is quite variable and varies from product to product. Therefore care should be taken when interpreting the data presented. Since so far no normalised extraction method for DNA is available, the development or acceptance of such a method becomes imperative.

In products derived from starch using technologies with a pronounced degradative impact on native starch, DNA is fragmented and PCR amplification is necessary in order to be able to detect DNA. However since the size of the fragments amplified can vary quite substantially *(*from 80 to over 700 base pairs) no consensus as to the optimal fragment size for amplification seems to exist. Observed differences among studies (i.e. detection versus absence of detection of DNA) may have as origin the differences in the DNA fragment size in the samples used for amplification.
Indications are[17] that the most reliable results are obtained with fragments having a size between 150 and 200 base pairs. 500 base pairs seems to be the absolute upper limit to obtain more or less reliable results.
These facts might limit the overall validity of the data presented above. It also shows the need for a validated analytical method for the detection of DNA.

Conclusions

Regarding “highly refined oils:

1. The term “highly refined oil” has not yet been unambiguously defined.

2. DNA and protein can be found not only in cold-pressed but in principle also in refined oils. The amounts in refined oils are however very low, but in certain cases detectable. Residual protein was detected in refined maize oils in different laboratories at a level below 100µg/ml. In the cases of extracts of oils derived from 100% GMO soybeans, transgenic DNA could be detected.

3. At present it is not possible to unambiguously specify which refining processes would ensure that DNA/protein are removed efficiently enough to be not detectable.

4. Some refining processes used by industry today may ensure that DNA/protein are efficiently removed. There is no guarantee however that these processes are commonly applied.

5. The information presently available does not allow to say what type of oils could be included in the list.

Regarding products derived from starch:

1. In native starch, protein is present in small amounts. The level of GMO protein can be estimated at 0.22 ppb.

2. In starch hydrolysates the level of CryIA(b) protein, (Bt-protein), can be estimated at 0.03 ppb. Such a value is considered to be below the limit of detection but presently no validated analytical methods are available to test the presence of these (new) proteins or fragments thereof.

3. In industrial samples of maize starch, maize DNA is present and can be detected.

4. No DNA has been detected in starch hydrolysates as final product or in samples taken at different stages of production.

6. Some refining processes used by industry today may ensure that DNA/protein are efficiently removed. There is no guarantee however that these processes are commonly applied.

The Committee wants to re-iterate that all novel foods accepted as being in accordance with the requirements expressed in its opinions regarding the assessment of novel foods are, on the basis of current knowledge, safe for human consumption. Therefore, the present issue is not related to health considerations but is only dealing with the legal element of labelling.

In general terms the Committee wants to emphasise that the potential detection of DNA and protein residues only depends on the sensitivity, precision and reproducibility of the presently available analytical methods.

The Committee also emphasises that any list of products in which neither protein nor DNA resulting from genetic modification is present must be subject to regular updating in the light of new analytical data derived from experimental work, as a result of enforcement investigations or from whatever relevant source.

Given the fact that only some refining processes used by industry today may ensure an efficient removal of either proteins or fragments thereof or DNA or fragments thereof and that therefore their absence can only be supposed, the Committee emphasises that the absence/presence of these

molecules should be confirmed by adequate analytical methods before the products are added or deleted from a list of products not requiring labelling with respect to genetic modification.

References

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