Determination of Common Wheat in Pasta Products Dried at Elevated Temperatures : An Update and New Perspectives

Jean-Claude Autran and Joëlle Bonicel
Laboratoire de Technologie des Céréales, I.N.R.A.
2 Place Viala, 34060 Montpellier, France

INTRODUCTION

Economic Background

Pasta are important foods in Europe, mainly in Italy (25 kg/capita/year), Greece, France (6 kg) and Spain. The best products (nutritional value, cooking quality, aspect) are processed from durum wheat semolina. Because of this difference in quality, and because of the new market regulation on January 1993, that will include a free circulation between the member States of pastas made of durum wheat / common wheat mixtures, the consumers will have to know through an efficient and verifiable labelling the exact composition of the pastas that are marketed.

In the past years, several methods were used for this purpose, especially electrophoretic or immunochemical determinations of proteins (albumins) or enzymes (peroxidases) specific of the genome D of common wheats. All these methods, however, are no longer reliable because of the introduction of technologies of pasta drying at elevated temperatures or of the development of other types of heat-treated products (couscous, precooked pasta).

Therefore, one or several other methods to determine the relative amounts of durum wheat and common wheat used in pasta manufacture had to be urgently developed that are compatible with the use of elevated temperatures in the pasta industries. It is the reason why the Commission of the European Communities (Community Bureau of Reference, DG XII) supported a three-year research programme (1990-1992) in view to explore, and to test through intercomparisons, several possible methods.

All these new methods are also based on protein components that are specific of the genome D of common wheats, but, unlike the previous methods, they must involve heat-resistant components, or at least take into account the denaturing effects of the new technologies.

Exposé présenté aux Journées du Blé Dur et des Pâtes Alimentaires (Durum- und Teigwaren Tagung), le 1° Avril 1992 à Detmold (Allemagne).

Four methods have been explored, plus a fifth one that was developed apart from the CEC programme, but that will be joined to the other methods when intercomparisons will be carried out.

In this report, we would like to give an overview of this CEC/BCR research programme, including a brief description of the various principles involved in the five methods investigated, and an outline of their respective interests, limitations and perspectives, in the aim of the common wheat determination in pasta dried at elevated temperatures:

1) Electrophoresis and quantitation of 1D-ω-gliadin fractions (Dr. Autran and Mrs Bonicel, Laboratoire de Technologie des Céréales INRA, Montpellier, France).

2) Reversed-phase high-performance liquid chromatography (RP-HPLC) of γ-gliadin fractions (Prof. Griffin and Mr. Barnwell, Nottingham Polytechnic, UK).

3) Determination of specific albumins ("A", "B", "C") of common wheat and ("1") of durum wheat (Prof. Resmini and Mr. Denoni, Università di Milano, Italie)

4) Immunoassay based on a monoclonal antibody produced against the *0.19* albumin of common wheats (Prof. Paraf, Laboratoire d'Immunologie INRA, Tours, France and Mr. Violle, Laboratoire de Technologie des Céréales INRA, Montpellier, France).

5) "Durum Test Immunoassay" based on a monoclonal antibody produced against the *friabilin* protein (Prof. Stimson, University of Strathclyde and Mr. Bony, Rhône-Poulenc Diagnostics, Glasgow, UK, and Lyons, France).

1) Electrophoresis and quantitation of 1D-ω-gliadin fractions (Laboratoire de Technologie des Céréales INRA, Montpellier, France).

Principle:

The research has focused on the ω-gliadin fraction that comprises the most heat-resistant proteins of the wheat kernel (because they are sulfur-free and therefore unable to form S-S bonded aggregates upon heating). After electrophoretic fractionation on a conventional polyacrylamide gel, the slow-moving triplet bands, that are specifically encoded by genes on the chromosome 1D of common wheat, may be used for common wheat determination. For that, these 1D-ω-gliadin bands may be either quantitated using a densitometer, or simply assessed by visual

examination on the basis of a set of standard mixtures. In addition, to erase the possible differences in the level of denaturation due to various drying conditions (and also to remove any background in the pattern and to restrict the analysis to the only ω–gliadin fractions), all samples are boiled during 15 min before extracting the gliadins.

Main results:

After extraction, electrophoretic fractionation and densitometric scanning of 1D-ω-gliadins:

- The densitometric response is linear for percentages of common wheat as high as 50 %; the general shape of the curves does not change whether surfaces or relative percentages of the ω-1D peaks are considered. For instance, very similar results were obtained for three sets of *Barilla* samples (drying temperatures: 60°C, 92° + 78°C and 100°C, respectively).

- A satisfactory measurement could be obtained very readily and at a much lower cost (without the need of a densitometer + software) by simple visual examination of the patterns, with reference to a set of standard mixtures.

- The method is applicable without any difficulty to even highly denatured products (cooked pasta, couscous).

- The experimental variation was estimated at ± 2 % for common wheat percentages below 10 %, at ± 3-4 % for common wheat percentages between 10 and 20 %; at ± 8-10 % for common wheat percentages between 20 and 40 %; at ± 15 % for common wheat percentages higher than 40 %.

- However, because the various cultivars of soft wheat do not contain the same amount of 1D-ω–gliadins, the varietal composition of a pasta sample is the main limitation to the method. The only possibility do deal with this question is to take into account the possible variation resulting from the varietal composition in the confidence limits of the results.

Comments:

Because of the influence of experimental conditions and varietal composition, this ω–gliadin method can be only semi-quantitative. For instance, it does not seem to have the capacity to significantly distinguish between more than five levels of adulteration (e.g. 0-6 %, 6-15 %, 15-30 %, 30-50 % and > 50 %). But, on the other hand, the great advantage of the method is to be applicable to any kind of heat-treated pasta, even highly denatured samples (dried pasta, precooked pasta, couscous), due to the extreme heat-resistance of ω-gliadins. In addition,

the method involves à conventional, well-described polyacrylamide gel electrophoresis. To date, no further work is needed: the method needs only to be tested by different technicians in different laboratories.

2) Reversed-phase high-performance liquid chromatography (RP-HPLC) of γ-gliadin fractions (Nottingham Polytechnic, UK).

Principle:

In this method, the detection of common wheat is based on another fraction of gliadins, which is identified through reversed-phase high-performance liquid chromatography (RP-HPLC).

RP-HPLC of hexaploid and durum wheat gliadins showed that peaks eluting between 47-49 min represented a fraction belonging to the group of γ-gliadins, that was also genome-D-specific proteins. The total peak area calculated from the elution profile at 206 nm forms the basis of determining the common wheat content of pasta samples. Furthermore, antisera were prepared using γ-gliadin isolated from this 47-49 min peak on RP-HPLC, so that the detection of common wheat could be alternatively possible by immunochemistry.

Main results:

- Taking advantage of the complete automatization and of the accurate quantitation facilities of the HPLC, this method allows detection of soft wheat adulteration of pastas at levels as low as 3-4 %.

- However, there are some limitations for this method too. While an accurate determination of the common wheat content from a set of *Barilla* samples seemed possible when pasta were dried at either 60°C or 92°+78°C, a much lower accuracy was observed from 100°C-dried samples. This difficulty was not totally resolved even after modifications of the solvent to obtain a better extraction of gliadins.

- In addition, as for the ω-gliadin method, difficulties resulted from variations in the varietal composition of the samples. Although no γ-gliadin peak was observed so far in any durum wheat sample, the 1D-γ-gliadin was found higher in certain cvs. such as Albatros than in other cvs. such as Camp Rémy.

Comments:

This RP-HPLC method has the potential for accurately detecting soft wheat adulterations of pastas at low levels and using an automatic and reproducible system. It involves, however, a costly equipment (RP-HPLC + computer software), as well as sequential analyses of samples (1 sample every 55 min, only). Furthermore, the effect of the highest temperatures and of the varietal composition are still not clearly resolved.

Considering the immunochemical (polyclonal antibodies) alternative method, specificity towards soft wheat gliadins was found to vary depending on the soft wheat variety and of the method of presentation of antigen to antibody, while the avidity of antibody was reduced in pasta samples compared with semolina.

3) Determination of specific albumins ("A", "B", "C") of common wheat and ("D1") of durum wheat (Università di Milano, Italie)

Principle:

Three albumin electrophoretic fractions ("A", "B", "C") were identified as specific of common wheat. Conversely, a durum wheat specific fraction ("1") was observed. Although these albumin fractions are denatured upon pasta drying at elevated temperatures, they are still partially extractable when using efficient solvents containing dissociating agents. Because there is a similar denaturation rate of "A", "B", "C" and "D1" proteins under heat treatments, the ratio (A+B+C)/D1 can be used to determine the common wheat content in a pasta sample, even if relatively high temperatures are used in drying.

Main results:

- Quantitation of these proteins is possible through an isoelectric focusing technique or, alternatively, by RP-HPLC, or immunochemical techniques (rabbit antisera).

- The relative proportions of common wheat and durum wheat specific fractions correlates well with the content of common wheat in durum wheat pastas.

- The results are not influenced by the varietal composition due to the presence of the A, B, C, and D1 albumin components in rather constant amounts in the various varieties of the two wheat species.

- It is possible to detect levels as low as 1.5% of common wheat but calibration curves are dependent of the drying conditions. In the case of

pastas dried at 100°C, no one of the albumin bands can be extracted, so that such 100°C-dried samples cannot be correctly analysed, despite of various modifications of the extraction and separation steps.

Comments:

The method has a high potential of accuracy for samples dried at low or moderately high temperatures. It has the great advantage to be independent of the varietal composition of the pasta sample. It cannot be used, however, with pastas dried at very high temperatures, or with those submitted to precooking treatments.

4) Immunoassay on albumin *0.19* (Laboratoire d'Immunologie INRA, Tours, and Laboratoire de Technologie des Céréales INRA, Montpellier, France).

Principle:

Two albumins specific of common wheat belonging to the family of α-amylase inhibitors have been purified. Monoclonal antibodies have been raised against one of them referred to as *alb 0.19.* Among these antibodies, some were found to react with the native form of the protein only whereas some others react with the denatured form only. Interestingly, one antibody could be selected that correspond to an epitope present on the native as well as on the denatured form of the protein. The latter has therefore the potential to recognise the specific albumin indiscriminately in its native or denatured state and to detect common wheat in pasta samples after any kind of heat treatment by a very simple and rapid ELISA immunoassay.

Main results:

- The conditions of solubilization of the protein were optimized to allow quantitative and reproducible extraction either for non-heated or heated samples.

- A sandwich ELISA test was developed based on the capture of the antigen by the monoclonal antibody, which is then revealed by a classical polyclonal serum.

- The specificity of this monoclonal antibody is however in question, some durum wheat cultivars being slightly recognised by the antibody.

Comments:

The use of specific monoclonal antibodies targeted against this albumin in heated pastas needs further development, including perhaps the production of another monoclonal raised against the denatured form of the protein, or the use of a combination of two different monoclonal antibodies: the first one (affected by drying temperatures but totally common wheat-specific) to detect the adulteration, and the second one (not especially common wheat-specific but not affected by drying temperature) to allow unknown samples to be referred to the correct standard curve.

5) Durum Test Immunoassay on *Friabilin* (University of Strathclyde + Rhône-Poulenc Diagnostics, Glasgow, UK)

Principle:

Monoclonal antibodies were raised against purified *Friabilin*, a 14.7K basic protein, encoded on chromosome 5D of hexaploid wheats, believed to be integral in the mechanism of hard/soft characteristic of wheat flour. A specific monoclonal antibody, coded as F7F, appeared to be specific of the S-S bridge C-terminal region of *Friabilin*. Durum wheats contain no *Friabilin*. Two tests were developed: a semi-quantitative dipstick test and an ELISA test.

Main results:

- Pure *Friabilin* was found sensitive to heat, but, in a mixture such as pasta, the other proteins seem to protect it against temperature so that no significant difference was found between *Buitoni* or *RHM* pasta standards treated during 0, 30, and 60 min at 100°C.

- A lower detection was observed, however, in pasta dried at 92°C + 78°C or 100°C than in those dried at 60°C or untreated. In 100°-dried pastas, *Friabilin* was still highly detectable, with lower response than at 60°C (jump between 60° and 92°). It is recommended to use standards dried at the same temperature.

- Some genetic variation was observed (cv. Norman contains less *Friabilin*), but no *Friabilin* was found in any of the 53 durum wheat cultivars tested.

Comments: This test is ready to be used. It has the considerable advantage of rapidity and simplicity, with high potential of sensitivity at low levels of adulteration. A full protocol is ready. The method can be submitted without any further work to intercomparisons.

CONCLUSION

To date, no one technique is available that can be used for the accurate determination of soft wheat in pastas dried at temperatures up to 100°C, especially when the highest temperatures are applied at the beginning of the drying cycle (high humidity).

Methods using wheat albumins have problems with drying temperature and sometimes with common wheat / durum wheat specificity, while those using gliadins are affected by genetic and environment variation.

Therefore, a compromise has to be found after considering again the exact request of the industries and the possibilities and limits of the present methods. Alternatively, a combination of methods may have to be used.

Any of the five techniques explored has a number of potential advantages but also of limitations resulting from either a change in the calibration curves at the highest drying temperature (100°C, or sometimes 92°C), or an insufficient specificity to soft wheat, or an influence of the varietal composition, as summarised in the following table.

	Specificity/ soft wheat	Indep./ variety	Indep./ 92°C	Indep./ 100°C	State of the protocol
ω–gliadin	+ + +	+	+ + +	+ +	+ + +
HPLC	+ + +	+	+ + +	+ +	+ +
IEF albumins	+ + +	+ + +	+ +	-	+
Mab/albumin 0.19	+	+ +	+ +	+	+ +
Durum Test/Friabilin	+ + +	+ +	+ +	+ +	+ + +

The ω-gliadin method seems to be the less sensitive to the highest temperatures, but it seems to be only semi-quantitative (indication of the range). Its protocol is ready, no further research work is needed.

The Durum Test immunoassay is also ready to be used. It seems to make a good compromise between independence to varietal composition and independence to drying temperatures, although there is some jump between 60°C and 92°C. A full protocol is ready.

Researches on RP-HPLC are essentially finished. The results seem significantly influenced by the varietal composition, but little influenced by the drying temperatures.The protocol will be ready in a few weeks.

These results tend to indicate that these three systems are now workable: they may be likely "to do the job". However, it is now necessary to check

through intercomparisons if the various laboratories find them suitable for their own use.

PERSPECTIVES

In 1992 the three following methods will be submitted to intercomparisons:

- Electrophoresis of ω-gliadins
- RP-HPLC of γ-gliadins
- Durum Test immunoassay.

The schedule should be the following:

- Identification of the labs (20 ?) who will participate to ring tests on a voluntary basis,
- Completion of the experimental protocols,
- Preparation of the samples: unknown pastas (different soft wheat percentages, different drying temperature and different varieties) will be tested in comparison with a reference set.
- Organisation of a two-day Workshop, presumably in Montpellier, France, to demonstrate and explain the three methods to all the participants to intercomparisons.

Each participant will then receive a protocol of the three methods, unknown samples, a set of standards and an analytical report sheet.

The results of the intercomparison will then be put in a report to be submitted to the Commission. The technique(s) giving the best satisfactory results will be put in the directives of the Commission.