Technological handbook of methods

Organisation name of lead contractors for this deliverable:
INRA Montpellier, INRAN Rome, Aarhus University.

Objectives of AGTEC-Org:

The overall objective of this project is to identify agronomical and food processing technologies that enhance the baking quality and the nutritional value of organic wheat and reduce mycotoxin contamination. Specific objectives are to:

• Evaluate the current practices for organic grain wheat production and flour-processing in Europe.
• Improve crop management strategies to enable bread-quality wheat to be produced on organic farms with and without livestock.
• Develop optimal post-harvest treatment to prevent mycotoxin contamination and enhance bread making quality and nutritional value.
• Generalise results from experiments in order to enhance farm management strategies in other climates and soil types.

The project is organized into five work packages and this deliverable deals with the WPs 3 and 4.

Objectives and description of WPs 3 and 4:

WP3. Post-harvest treatments (INRA, INRAN, Goëmar)
Grain samples from WP2 (field experiments) varying in DON and vitreousness degree will be selected. Milling tests will be carried out with either roller milling or stone milling using three extraction rates: 70, 80 and 90%. Flour will be analysed for its technological, nutritional and hygienic quality (see WP4) and the optimal extraction rate will be determined. Pre-treatments (dehulling and ozonation) and post-physico-chemical treatments (heat treatments) suitable for organic processing will be tested by INRA in collaboration with Goëmar Industry. These complementary treatments will be studied on samples selected from WP2 that exhibit a high level ofDON with different baking values, vitreous and floury kernel texture. Detailed attention will be paid to the characterization of the redox status of flour components in relation to dough rheological properties (proteins) and nutritional properties (antioxidant potential). Finally, a combination of different complementary treatments will be studied in order to define optimal conditions to improve flour characteristics.

WP4. Grain and flour quality (INRA, INRAN, DIAS)
Grains collected in the field experiments (WP2) and exposed for different pre and post-treatments and milling techniques (WP3) will be provided. Analyses of hardness, ash, total protein, dietary fibre, bound hydrophilic antioxidants will be performed together with some specific physico-chemical parameters for proteins such as Zeleny sedimentation index and gluten index and flour rheological properties (farinograph, alveograph, gluten index, extensibility test, sedimentation test). Phytate content and protein composition (size of protein polymers, gliadin to glutenin ratio, HMWG to LMWG ratio) will be performed together with analyses of granulometry and damaged starch and DON contamination. INRA and INRAN will take care of the technological and nutritional evaluation, and DIAS will take care of the safety aspects by performing analyses of the DON.

Specific objective of this handbook:

This handbook is a working tool for scientists involved in the WPs 3 and 4. It contains the protocols used for sampling, sample preparation, processing of grains and for analysing grains and flours and it is composed of sheets, each dealing with a different topic.

Sheet No 1

1. Sampling and sample preparation

2. Work Packages: WP3 and 4.

3. Objective: The principle of sampling is to obtain an average sample of grain or flour corresponding in every respect of the average characteristics and composition of the field, lot, parcel, etc. from which it has been drawn

4. Literature:

ICC Standard methods of the International Association for Cereal Science and Technology. Standard No. 101/1.ICC, Vienna, Austria, 2003.

AACC 2000 Approved methods of the American Association of Cereal Chemists, 10th ed. Methods 44-15 A, 44-40. The Association, St. Paul, MN, US.

5. Procedure:

.Samples shall be fully representative of the lots from which they are taken. A representative sample is essential for a meaningful quality analysis. Therefore, as the composition of the lot is seldom uniform, a sufficient number of increments shall be taken and carefully mixed, thus giving a bulk sample from which are obtained, by successive divisions, the laboratory samples.

The location and time of sampling shall be determined by agreement between the parties concerned.

Samples of unsound material shall not be mixed with samples of the sound material.

Special care is necessary that all sampling apparatus is clean, dry and free from foreign odours. Sampling shall be carried out in such a manner as to protect the samples, the sampling instruments and the containers in which the samples are placed from adventitious contamination such as rain, dust, etc.

Sampling from bulk can be made with shovels, hand-scoops, cylindrical samplers and apparatus for taking increments periodically from a flow of grain. Sampling from bags can be done with sack-type spears or triers and increments shall be taken from different parts of a bag (for example top, middle and bottom).

The bulk sample shall be formed by combining the increments and mixing them well. The bulk sample shall be divided to obtain the required number of laboratory samples. The number of laboratory samples to be taken for analysis shall be specified by mutual agreement.

Also the size of samples will be defined depending on the quality tests to be performed.

The laboratory samples must not contain extraneous matters such as stones, soil, other vegetable matter, etc. They shall be packed in clean, possibly new, bags of cotton or of other synthetic material of very close texture, or of strong paper. It is important that each bag is adequately sealed to prevent the escape of sample during travelling.

Each sample must be adequately labelled and care must be taken that the information recorded on the label shall be permanent and the label remains with the sample all the time to avoid confusion between samples.

Laboratory samples shall be dispatched as soon as possible after sampling.

A sampling report should be prepared by the sampler for each batch of samples dispatched for analysis making reference to the condition of the grains sampled, the technique applied if different from these guidelines and all the circumstances that may have influenced sampling.

Sheet No 2

1. Evaluation of grain and flour ash content

2. Work Packages: WP3 and 4.

3. Objective: to analyse ash content of wheat grains grown under different cropping systems and their derived flours in order to evaluate their technological potential and as an estimation of their fibre content.

4. Literature: ISO Method No. 2171

5. Procedure:

Incineration in muffle. In the case of grains they are previously milled in a suitable laboratory mill (such as Bühler MLI 204 disk laboratory mill) to produce a wholemeal flour. An aliquot of about 5g is accurately weighted in a platinum bowl which is placed in a muffle and incinerated at a temperature of 900 ± 25°C °C (for wholemeal flour from grains) or 550 ± 10 °C for flours. The residue is cooled in a desiccator and weighted.

6. Requirements: ca 20 g wheat grain or flour

7. Information on:

- Wheat variety
- Cropping system
- Processing

8. Handling:

— Grain samples should be thoroughly cleaned and free from foreign seeds, stones and other vegetable matters.

— Labelling of samples should be described

— Send samples to:

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Sheet No 3

1. Evaluation of grain and flour moisture content

2. Work Packages: WP3 and 4.

3. Objective: to analyse moisture content in wheat grains grown under different cropping system and in their flours to be able to express results of other quality tests on dry matter.

4. Literature: ICC Standard methods of the International Association for Cereal Science and Technology. Standard No. 110/1.ICC, Vienna, Austria, 2003.

5. Procedure:

Oven drying at 105 °C until constant weight or at 130 °C for 90 min. In the case of grains they are previously milled in a suitable laboratory mill (such as Bühler MLI 204 disk laboratory mill) to produce a wholemeal flour. An aliquot of about 10 g is accurately weighted in a moisture bowl (glass, porcelain or plastic) which is placed in an electric drying oven at the chosen temperature. The dried flour is cooled in a desiccator and weighted.

6. Requirements: about 20 g

7. Information on:

- Drying after harvest
- Cropping system

8. Handling:

- Grain samples should be thoroughly cleaned and free from foreign seeds, stones and other vegetable matters.
- Labelling of samples should be described.
- Send samples to:

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Sheet No 4

1. Evaluation of grain and flour protein content

2. Work Packages: WP3 and 4.

3. Objective: to analyse protein content in wheat grains grown under different cropping system and in their flours in order to predict their baking potential.

4. Literature: ICC Standard methods of the International Association for Cereal Science and Technology. Standard No 105/2, ICC, Vienna, Austria, 2003.

5. Procedure:

Protein content is calculated by multiplying the total content of nitrogenous compounds of the analysed product by a conventional factor which is 5.70 for soft wheat. In particular the organic matter present in about 1 g of accurately weighted flour (in the case of grains they are previously milled in a suitable laboratory mill such as Bühler MLI 204 disk laboratory mill or 1093 Sample Mill, Cyclotec, Tecator, to produce a wholemeal flour) is oxidized with concentrated sulphuric acid in the presence of a catalyst: the product of the reaction containing ammonia is treated by alkali; free ammonia is distilled and titrated

6. Requirements: ca 5 g wheat grains or flour

7. Information on:

- Wheat variety
- Cropping system
- Processing

8. Handling:

- Grain samples should be thoroughly cleaned and free from foreign seeds, stones and other vegetable matters.
- Labelling of samples should be described.
- Send samples to:

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Sheet No 5

1. Evaluation of grain and flour protein quality by means of the Zeleny Sedimentation Test

2. Work Packages: WP3 and 4.

3. Objective: to analyse protein quality in wheat grains grown under different cropping system and in their flours in order to predict their baking potential

4. Literature: ICC Standard methods of the International Association for Cereal Science and Technology. Standard No 105/2, ICC, Vienna, Austria, 2003.

5. Procedure:

The degree of sedimentation of flour suspended in a lactic acid solution during a standard time interval is taken as a measure of baking quality. Swelling of the gluten fraction of flour in lactic acid solution affects the rate of sedimentation of a flour suspension in the lactic acid medium. Higher gluten content and better gluten quality both give rise to slower sedimentation and higher Sedimentation Test values. In particular, 3.2 g of accurately weighted flour (14% moisture basis) (in the case of grains they are previously milled in a suitable laboratory mill such as Sedimat 880502, Brabender) are placed in a graduated cylinder, reagents are added and mixed thoroughly. After mixing with all reagents, the cylinder is left to stand for exactly 5 min and the volume of sediment is read. Values below 20 ml indicate a poor baking quality.

6. Requirements: ca 30 g wheat grains or flour

7. Information on:

- Wheat variety
- Cropping system
- Drying after harvest

8. Handling:

- Grain samples should be thoroughly cleaned and free from foreign seeds, stones and other vegetable matters.
- Labelling of samples should be described.
- Send samples to:

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Sheet No 6

1. Evaluation of grain and flour gluten quantity and quality by means of the Gluten Index Method

2. Work Packages: WP3 and 4.

3. Objective: to analyse gluten quantity and quality in wheat grains grown under different cropping systems and in their flours in order to predict their baking potential.

4. Literature: ICC Standard methods of the International Association for Cereal Science and Technology. Standard No. 155, ICC, Vienna, Austria, 2003.

5. Procedure:

Wet gluten, a viscoelastic substance made of gliadin and glutenin, is mechanically separated from the flours (10 g accurately weighted to 0.01 g) by means of the Glutomatic equipment under standardized conditions. The wet gluten is then centrifuged to force it through a specially constructed sieve under standardized conditions. The total weight of the gluten is defined as gluten quantity. The percentage of wet gluten remaining on the sieve after centrifugation is defined as the Gluten Index. If the gluten is very weak all of the gluten may pass through the sieve and the GI is then 0. When nothing passes, the Index is 100.

In the case of grains a whole wheat meal is prepared by grinding wheat in a Falling Number Laboratory Mill 3100 or 120 equipped with a 0.8 mm sieve.

6. Requirements: ca 30 g wheat grains or flour

7. Information on:

- Wheat variety
- Cropping system
- Drying after harvest

8. Handling:

- Grain samples should be thoroughly cleaned and free from foreign seeds, stones and other vegetable matters.
- Labelling of samples should be described.
- Send samples to:

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Sheet No 7

1. Evaluation of flour baking quality by means of the Brabender Farinograph

2. Work Packages: WP3 and 4.

3. Objective: to analyse the mixing behaviour of a dough coming from wheat flour obtained from grains grown under different cropping systems in order to predict their baking behaviour.

4. Literature: ICC Standard methods of the International Association for Cereal Science and Technology. Standard No. 115/1, ICC, Vienna, Austria, 2003

5. Procedure:

The Brabender Farinograph measures (as torque) and records the resistance to mixing of a dough as it is formed from flour and water, developed and broken down. The maximum consistency of the dough is adjusted to a fixed value by altering the quantity of water added. This quantity, the Farinograph water absorption, is used to obtain a complete mixing curve, the various features of which are a guide to the strength of the flour. In particular, 300 g of flour weighed to the nearest 0.1 g and having a moisture content of 14% are mixed with a volume of water to give a maximum consistency of approximately 500 FU. The dough is kept mixing for 20 min from the addition of water during which a curve is registered which is then used to calculate farinographic parameters (dough development time, dough stability, degree of softening, quality number).

6. Requirements: about 1kg of flour

7. Information on:

- Wheat variety
- Cropping system
- Drying after harvest
- Processing

8. Handling:

- Labelling of samples should be described.
- Send samples to:

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Sheet No 8

1. Evaluation of flour baking quality by means of the Chopin-Alveograph

2. Work Packages: WP3 and 4.

3. Objective: to analyse the rheological properties of dough coming from wheat flour obtained from grains grown under different cropping systems in order to predict their baking potential.

4. Literature: ICC Standard methods of the International Association for Cereal Science and Technology. Standard No. 121, ICC, Vienna, Austria, 2003

5. Procedure:

A dough from flour, salt and water is prepared under standard conditions. The dough is formed into disc-shaped pieces and after a fixed resting period the pieces are inflated into bubbles. The pressure variation inside each bubble is recorded in a graph. These pressure variations describe the resistance to stretching and the extensibility of the dough tested. The length and the shape of the curve obtained by the extension of the bubble at the moment of rupture are the criteria of the physical properties of a dough and hence of the baking characteristics of the flour. In particular, the equivalent of 250 g of flour of 15.0 % moisture content are weighed to the nearest 0.5 g and placed in the mixer, the required volume of sodium chloride solution is added and the dough is formed. The dough is extruded and the test pieces are prepared which are left to rest. The test is then started 28 min after the mixing began. The following rheological parameters are derived from the curve: height of the curve H, length of the curve L, area under the curve S, deformation energy W, the ratio P/L.

6. Requirements: about 300g of flour

7. Information on:

- Wheat variety
- Cropping system
- Drying after harvest
- Processing

8. Handling:

- Labelling of samples should be described.
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Sheet No 9

1. Evaluation of grain and flour soluble, insoluble and total dietary fibre content

2. Work Packages: WP3 and 4.

3. Objective: to analyse dietary fibre quantity in wheat grains grown under different cropping systems and in their flours as an indication of their nutritional quality.

4. Literature: AACC 2000 Approved methods of the American Association of Cereal Chemists, 10th ed. Method 32-07. The Association, St. Paul, MN, US.

AOAC 2000 Official Methods of Analysis of the Association of the Official Analytical Chemists, 17th edition, Method 991.43. The Association, Arlington, USA.

Prosky L., Asp N.G., Schweizer T.F., De Vries J. W. and Furda I. 1988 Determination of insoluble, soluble and total dietary fiber in food and food products: interlaboratory study. Journal Association Official Analytical Chemists.

5. Procedure:

Dietary fibre, i.e. the remnants of the edible part of the plant that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the human large intestine, is measured by an enzymatic-gravimetric method. The main steps include enzymatic treatments for starch and protein removal, precipitation of soluble dietary fibre components by aqueous ethanol, isolation and weighing of the dietary fibre residue and correction for protein and ash in the residue. The enzyme digest can be filtered before the alcohol precipitation to recover insoluble fibre separately. The soluble fibre is then precipitated from the filtrate and recovered in a separate fraction. In the case of grains they are previously milled in a suitable laboratory mill such as 1093 Sample Mill, Cyclotec, Tecator, with a 0.5 mm sieve, to produce a whole meal flour. In particular the analysis is performed on aliquots of 1.000 0.005 g.

6. Requirements: about 20 g grains or flour

7. Information on:

- Wheat variety
- Cropping system
- Processing

8. Handling:

- Grain samples should be thoroughly cleaned and free from foreign seeds, stones and other vegetable matters.
- Labelling of samples should be described.
- Send samples to:

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Sheet No 10

1. Evaluation of grain and flour hydrophylic insoluble bound antioxidants (phenolic acids)

2. Work Packages: WP3 and 4.

3. Objective: to analyse insoluble bound phenolic acids in wheat grains grown under different cropping systems and in their flours as indicators of their antioxidant potential.

4. Literature: Carcea, M., Bruschi. L., and Quattrucci E. 2001 Influence of processing on the levels of some hydrophilic antioxidants in cereal kernels" Proceedings of the 11th International Cereal and Bread Congress, Cereals 2000, Published by the Cereal Chemistry Division, Royal Australian Chemical Institute, Melbourne, Australia, 216-219.

Decker E., Beecher G., Slavin J., Miller H.E., Marquart L. 2002 Whole grains as a source of antioxidant. Cereal Foods World, 47 (8), 370-373.

Hatcher, D.W. and Kruger, J.E. 1997 Simple phenol acids in flours prepared from Canadian wheat: relationship to ash content, color and polyphenol oxidase activity. Cereal Chem., 74(3), 337-343.

5. Procedure:

Phenolic acids (ferulic, sinapic, coumaric, vanillic, caffeic, etc. acids) are common hydrophilic antioxidants that are ubiquitous in fruits, vegetables, legumes and grains In grains in particular they are responsible for most of their antioxidant potential. Phenolic acids of wheat and other cereals are in the free, soluble esterified and insoluble bound forms and they are mainly present in the bran layer: the insoluble-bound forms make up around 80 % of the total. This fraction can be quantified in the ground sample (about 15 g accurately weighed) by extracting it firstly with acetone/water and then with methanol/water. The solid residue after centrifugation undergoes an alkaline hydrolysis and acidification, followed by a solid phase extraction (C18). The insoluble bound phenolic acids are then analysed by an HPLC system consisting of a reverse phase Supelco LC-18 analytical column at room temperature and a UV Detector (260 nm). In the case of grains they are milled with a suitable laboratory mill (such as Analysenmühle A10 Janke & Kunkel, IKA LABORTECHNIK, Germany) to produce a wholemeal flour.

6. Requirements: about 30 g grains or flour

7. Information on:

- Wheat variety
- Cropping system
- Processing

8. Handling:

- Grain samples should be thoroughly cleaned and free from foreign seeds, stones and other vegetable matters.
- Labelling of samples should be described.
- Send samples to:

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Sheet No 11

1. Evaluation of mycotoxin contamination

2. Work Packages: WP3 and 4.

3. Objective: to analyse insoluble bound phenolic acids in wheat grains grown under different cropping systems and in their flours as indicators of their antioxidant potential.
4. Literature: http://www.fooddiagnostics.dk/ProSmart/dox/PDF/R5906_td.pdf
5. Procedure:
Grains (ca 20 g) are milled in a laboratory mill (IKA-Werke A10) for 60 sec to produce a wholemeal flour. After thoroughly mixing, a portion of 5.00 g flour is transferred to a Falcon tube with 25 ml of distilled water. The cylinder is shaken on a Janke & Kunkel KS 501 D for 10 min at 225 rpm and centrifuged 10 min at 3000 g. An aliquot of 50 µl of the filtrate is transferred to a well in a test kit (Ridascreen® DON R5906, R-Biopharm, Germany) and treated according to the manufacturers instruction. Two replicate subsamples are prepared from each flour sample and two analyses are run for each subsample. The remaining flour (ca 10 g) is divided into two portions and oven dried at 130°C for 2 h for determination of dry matter content.
6. Requirements: about 50 g grains
7. Information on:
- Wheat variety
- Cropping system
- Processing

8. Handling:
- Grain samples should be thoroughly cleaned and free from foreign seeds, stones and other vegetable matters.
- Labelling of samples should be described.
- Send samples to:
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