THE IMPROVEMENT OF THE PROTEIN QUALITY OF SORGHUM AND ITS INTRODUCTION INTO STAPLE FOOD PRODUCTS FOR SOUTHERN AND EASTERN AFRICA: INCO DC CONTRACT 1C18-CT96-0051

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This project, which had two European partners and three African Partners, was funded under the INCO-DC programme of the 4th Framework. The overall objectives of the programme were to identify the factors adversely affecting the digestibility of sorghum proteins, to devise routes to improve sorghum digestibility to show how traditional technologies such as malting and fermentation affect digestibility, to investigate how nutritionally improved products could be used as ingredients in staple foods such as bread and weaning foods.

The outcomes of the project were to show that a number of factors affected the digestibility of the proteins, but that the most likely cause was the change in resistance to enzymic attack of the outer protein coat of the protein body after cooking. Malting and fermentation improve digestibility. In malting the project showed that the use of alkaline steeping could dramatically improve malting quality. Both malted and fermented foods could be successfully incorporated into bread and extruded weaning foods and have been found to produce consumer acceptable products.

The results of the research were disseminated by workshops in South Africa and Kenya and summarised in handbooks published in Portuguese and English.

INTRODUCTION

This project ran from 1996 to 1999. It arose out of a concern that although sorghum is crop well suited to growing in southern and eastern Africa it is not as well exploited as it might be and its nutritional potential was not being realised because of the low digestibility of the protein content of the grain. The general approach was to try to understand the basis of the low digestibility and to explore the ways that traditional methods such as malting and fermentation could be used to improve the protein digestibility. In order to ensure that the nutritionally improved products could be utilised it was also decided that attempts should be made to incorporate them in staple foods such as bread and weaning foods.

There were a total of six partners with three African and two European countries participating in the project (see Table I). A feature of the programme was high level of exchange and training with partners from Mozambique and Kenya registering at the University of Pretoria for PhD degrees. Visits were also made to the UK and Portuguese partners for training in a number of techniques. The programme was also supplemented by a Commonwealth Universities Studentship awarded to the, then, Mr Gyebi Duodo to work on the digestibility problem in the UK

The objectives of the project were to:

- Quantify and identify using enzymic and NMR and FTIR techniques, the factors adversely affecting the digestibility of sorghum proteins and to show how the traditional technologies of malting and fermentation affect the digestibility of proteins

- On the basis of the information obtained to devise routes to improve protein digestibility and how fermentation and malting technologies could be improved to offer better protein digestibility.

- To investigate how nutritionally products could be used as ingredients in staple foods such as bread and weaning foods.

- To exploit the results with a view to improving the economic status of people in sub Saharan Africa

These are ambitious objectives and in retrospect were too ambitious for a project that only had 6 partners collaborating for three years. However considerable progress was made and an important out come has been the creation of a network in Africa and between Africa and Europe that has proved fruitful.

The project was divided into 9 work packages which are listed in Table II
In order to be effective there had to be continual flow of material and information around the system shown in figure 1. In order to reduce costs we had originally decided to have meetings only once at year, in fact these turned out to be too infrequent so another meeting, at the 18-month stage was organised. We also made use of regular reporting by email.

INSTITUTION	LEADER	ABBREVIATED NAME
Institute of Food Research Norwich research Park Norwich NR4 7UA UK	Prof. PS Belton (coordinator)	IFR
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Table I The Partners in the Project

Work Package number	Work Package Title	Responsible partners
WP1	Provision of Sorghum Varieties	CSIR, UEM, KIRDI, UP
WP2	Dehulling ,Protein isolation and Purification	CSIR, UP, KIRDI
WP3	Enzymatic assays	UP
WP4	Fourier Transform Infrared Studies	UA, IFR
WP5	Nuclear Magnetic Resonance Studies	IFR
WP6	Malting	CSIR, UP
WP7	Secondary Processing	CSIR, UEM, UP, KIRDI
WP8	Consumer acceptability	UEM, UP, KIRDI
WP9	Transfer of Technology	ALL

Table II The Work Packages

THE WORK PROGRAMME

The first stage of the work was to source the sorghum from typical local varieties in Mozambique, South Africa and Kenya and then prepare them for use. Only condensed tannin free varieties, which were considered to be of good germinability, were used. The grains were stored in cold storage until ready for use. Milled samples were also produced after a dehulling procedure. Protein fractionation was carried out using the Osbourne's method as adapted by Taylor[1]. This method discriminates between proteins which are soluble without reduction of disulphide bonds, the P1 fraction, proteins which are soluble only on addition of a disulphide bond reducing agent, the P2 fraction and proteins that are not soluble at all, the residue. In sorghum wet cooking caused a great reduction in the P1 fraction, an increase in the P2 fraction and an increase in the residue. This contrasts with the more digestible maize where cooking caused only a small increase in the P1 fraction and a slight decrease in the residue. It is significant that the low digestibility of sorghum protein is only manifested after wet cooking[2] not after dry cooking processes such as popping[3].

The effects of cooking on the protein profile have been explored in some detail by SDS PAGE gel electrophoresis[2] and spectroscopy[4]. Generally the effects of cooking on the protein profile are relatively small. However Pepsin-indigestible residues from protein-body enriched samples consisted mainly of α-kafirin in uncooked sorghum, whilst cooked sorghum had in addition, γ- and β-kafirin and reduction-resistant 45–50 kDa oligomers.

Investigation of the proteins using infrared spectroscopy[4] showed that their structure was mainly α helical with a small proportion of β sheet. This is similar to the structures observed for the homologous protein from maize, zein. On cooking there was shift in

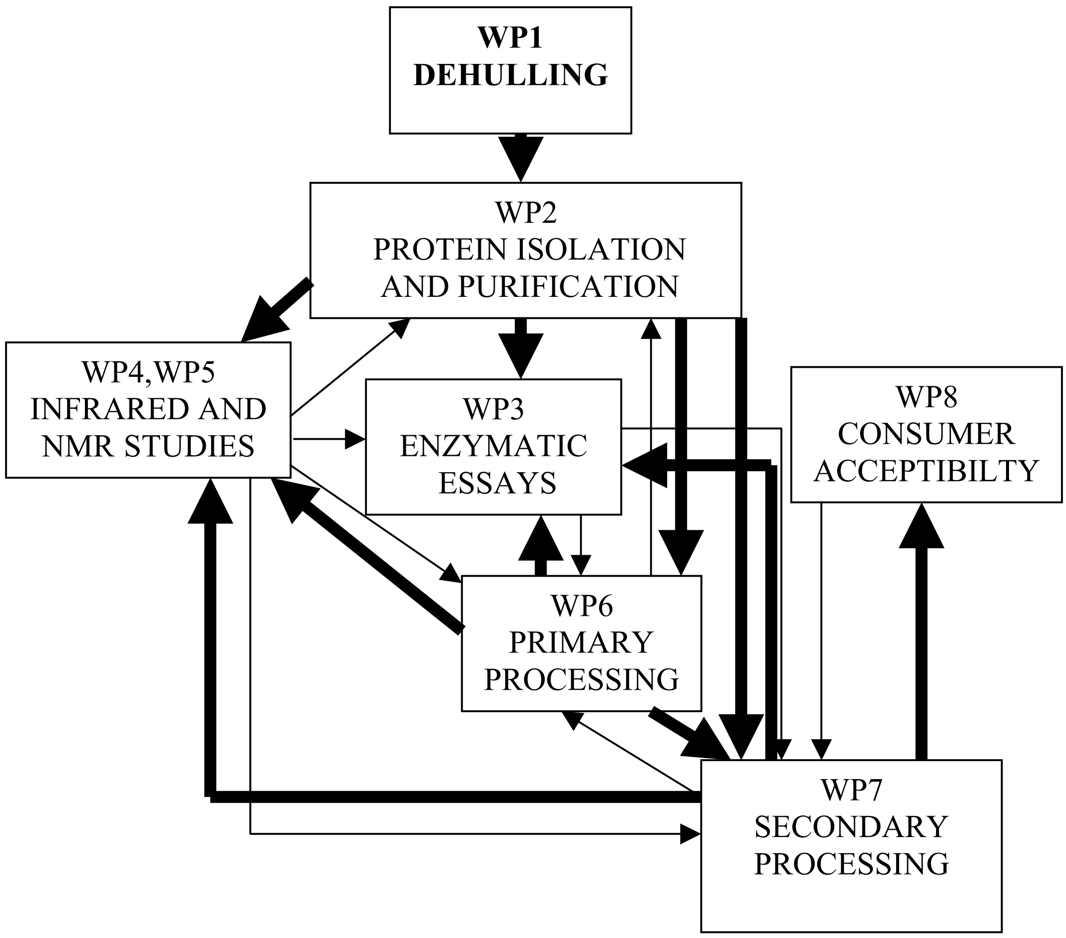

Figure 1 Flows of information (thin arrows) and material (thick arrows) between the work packages.

structure with a decrease in the amount of α helix present and increase in the amount of β sheet present. This is consistent with partial structural loss associated with some degree of coagulation of the proteins. Typical spectra are shown in Figure 2. Similar results are seen with zein but in this case the degree of β sheet formation is less.

Changes in water absorption as the temperature changes are an indication of the relative hydrophobicity of a protein[5]. The changes may be observed by using an NMR technique [5,6]. Figure three shows the effects of cooking on the amount of excess water in a saturated kafirin system when temperature is varied. Clearly when kafirin is cooked there is a significant change in its ability to absorb water. In both cases kafirin absorbs more water as temperature is increased indicating that it is hydrophilic, however heating

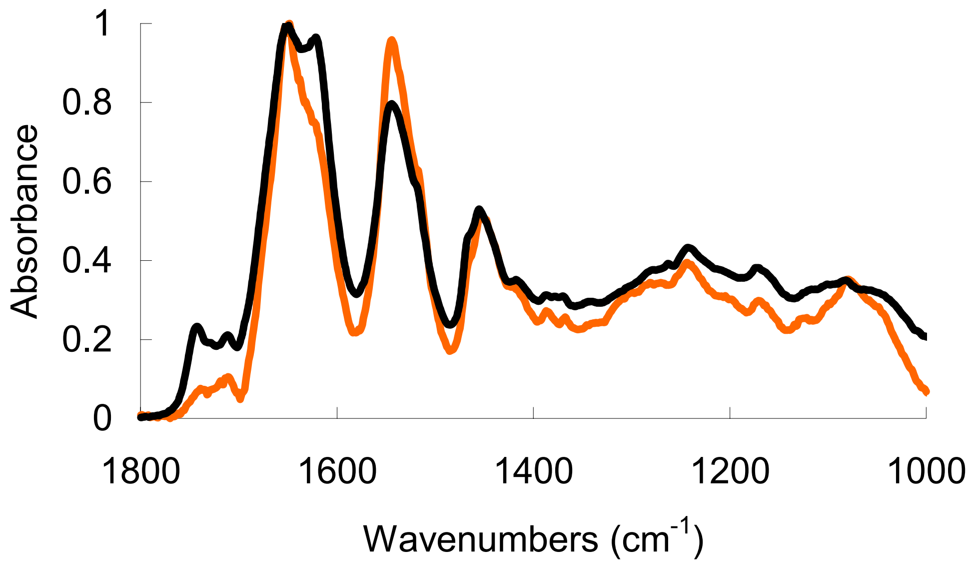

Figure 2 A partial Infrared spectrum of cooked (black line) and uncooked (red line) kafirin

reduces the absorption capacity and might therefore be considered as indicative of crosslinking, which would reduce swelling capacity. However similar effects are also seen with zein so that if this is occurring in kafirin it is also occurring in zein, but zein does not lose its digestibility on cooking.

The details of enzymic digestibility studies are dealt with elsewhere in this proceedings. The outcome of the experiments was to show that the main cause of indigestibility was wet cooking[2]. However as yet the cause of the indigestibility remains unclear since most of the possible explanations of the indigestibility proposed so far would apply also to maize (for a recent review see ref 7).

Processing of sorghum

Malting of sorghum improved both the protein quality and digestibility. Generally speaking the higher the quality of the malt produce the better was the protein quality and digestibility. An important discovery mad during the course of the research programme was that steeping the grain in a dilute solution of sodium hydroxide for 8 hours of a 24 hour steeping period improved the quality of the malt dramatically[8].

The improvement in diastatic power was 44% in optimum conditions. This finding is of great significance to the brewing industry and will be of value in the development of any malting process. However the optimum concentrations of sodium hydroxide are affected by variety and may be seasonally dependent so that careful work is required before the method is used. Fermentation also improved protein digestibility. Combinations of cooking and fermentation in any order resulted in improved digestibility.

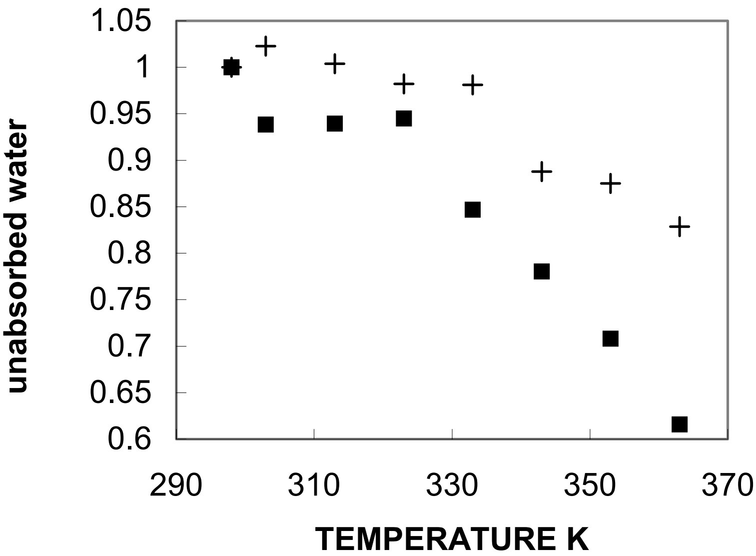

Figure 3 A plot of the water sorption of kafirin before (squares) and after cooking (crosses)

The origins of this effect are probably in both the enzymic breakdown of the proteins by the microorganisms in the fermentation medium and the effects of the decrease in pH during fermentation (pH 6.4-6.6 dropping to pH 3.7)

One of the objectives of the project was to incorporate sorghum in staple foods. Two examples were chosen as being both valuable in their own right and as typical of different processing technologies. The first example was a weaning food, which it was hoped, would provide a more nutritious product than the traditional sorghum based weaning food. In weaning foods, as far as protein is concerned, there are two criteria which must be met, the protein content per unit volume must be adequate for the nutrition of the child and the viscosity must be low enough for the child to be able to eat it.

The manufacture of an instant weaning food that could be simply reconstituted by the addition of milk or water was undertaken in a two stage process: a variety of mixtures of untreated, fermented and malted sorghum flours were extruded and then roller milled. The mixtures used are listed in Table 3. The addition of malted material seems to decrease the viscosity, the same is true for the addition of fermented material but in this case the effect is smaller. The effects on digestibility are less evident. This should be compared with the results on obtained for the raw and cooked fermented and malted materials where a significant difference was observed. However the process of extrusion and milling is likely to change the digestibility of the untreated sorghum flour. The important factor in these procedures is the drop in viscosity which is achieved by adding malted or fermented material. Where viscosity is low additional solids may be added. These will increase the viscosity, but since the desirable viscosity is below about 3000 cP there is some room for increasing solids without making the food unpalatable.

Ratio of components[a]	Protein content[b]	*In vitro* protein digestibility[c]	viscosity[d]
1:0:0	11.3	55.8	3584
25:75:0	11.3	60.2	2590
50:50:0	11.5	57.4	2498
75:25:0	11.6	53.5	2527
25:0:75	11.2	56.3	3441
50:0:50	11.1	55.9	3264
75:0:25	11.2	64.3	2944
50:25:25	11.3	59.3	2827
25:50:25	11.2	58.0	2567
25:25:50	11.2	58.9	2364

a the ratio is given in weight per weight of plain flour: malted sorghum: fermented sorghum. b gm/100gm of dry weight .c expressed as a percentage of total protein
d in centipoises

Table III The properties of instant weaning foods

In order to try and increase the consumption of sorghum, which unlike wheat can be locally produced by traditional methods, sorghum was incorporated into bread formulations[9,10]. In addition to plain sorghum flour both malted and fermented sorghum flours were added. Effort was concentrated on the addition of malted sorghum to the bread as this proved to be the most acceptable to consumers.

A formulation was used which incorporated 25% by weight of sorghum flour and 75% by weight of wheat flour. The way in which the malted flour is treated flour had a very strong effect on behaviour of the bread. This is shown in Table IV. Interestingly the behaviour of the dried malts together with the wheat flour and sorghum flour shows a strong correlation between loaf specific volume and holding viscosity. This breaks down for the malts heated in contact with water or water vapour before drying.

Overall the best conditions for making an acceptable sorghum containing bread were found to be by incorporation of 30% boiled malt sorghum. This gave a soft product which was more resistant to firming and a better taste and chewing quality. The important factors in determining the suitability of a malt product for incorporation into bread was complete inactivation of amylases and higher contents of gelatinised starch[10].

Malt drying temperature (°C)	Loaf volume (cm^3)	Loaf weight (gm)	Specific volume (cm^3/gm)
50	1776	822	2.1
80	1875	820	2.2
100	1858	811	2.3
120	2067	806	2.6
150	2067	801	2.6
Unmalted sorghum flour added	2708	796	3.4
100% wheat flour	3567	739	4.9

Table IV The properties of bread made from sorghum flour

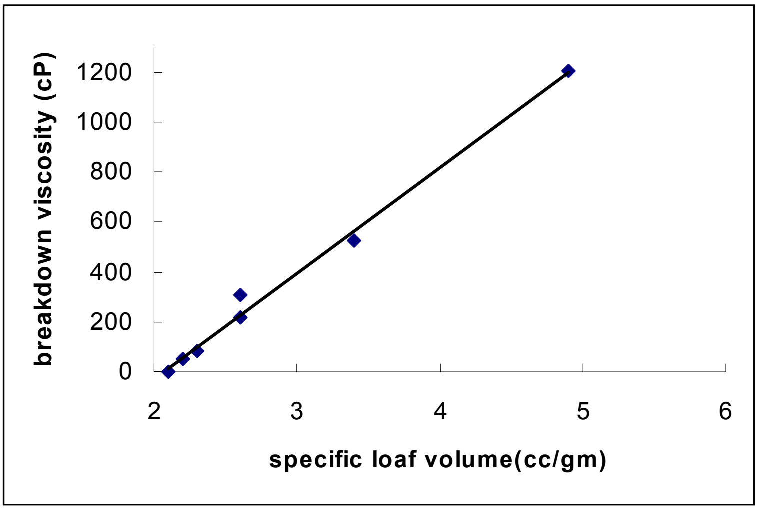

Figure 4 The variation of the specific loaf volume with the breakdown viscosity of the sorghum malts

Sensory testing

No food, no matter how worthy, will meet general acceptance unless it meets a number of requirements. It must be socially and culturally acceptable and it must be palatable.

Sorghum based foods meet the first two criteria but testing palatability was important. Tests were carried out on the weaning food in South Africa and Kenya and on the bread in Mozambique. It could not be assumed that all the consumers on the panels were literate and therefore suitable means of indicating preferences had to be utilised. Typically these were smiling or frowning stickers to indicate like or dislike. The testing process was introduced and explained in the appropriate local dialects before the testing process began.

Whilst consumers liked both the bread made with malted flour and with untreated sorghum flour on the basis of a tasting session, they did not favour the weaning food made with malt. Instead products made from untreated sorghum flour or fermented materials or a mixture of fermented and malted were preferred.

Dissemination

The outcomes of the project were disseminated in a variety of ways. In order to ensure the scientific quality of the project it is important to publish papers in peer-reviewed journals. This is also the best way of communicating with he scientific community. However it is important to realise that the audience for such a project is much wider than the scientific community and that other forms of communication are necessary. In order to be able to disseminate information to a wider audience, workshops, organised by UP and KIRDI, were held in South Africa and Kenya. These were attended by a mixture of local businesses, academia and community groups. In 1998 UP hosted a four day workshop sponsored by INSTORMIL (the international sorghum and millet collaborative research support programme of USAID) in which all the African participants of the project were present. There have also been numerous other oral presentations at conferences.

A further step was taken by producing handbooks in Portuguese and English entitled “Information handbook: sorghum malting, sorghum and fermentation, preparation of composite bread and instantised weaning food”, which included contributions from UEM, UP and KIRDI. The book provided a straightforward guide to methods of preparation appropriate on the small business, and in some cases, domestic scale.

Prof Taylor and Prof Belton organised a meeting under the auspices of the ICC (International Association for Cereal Science and Technology) entitled Challenges in Speciality Crops. As a result a proceedings was published under the title “ Increasing the utilisation of sorghum, buckwheat, grain amaranth and quinoa for improved nutrition”. An outcome of this was an invitation by Springer to Profs Taylor and Belton to edit a book entitled “Pseudocereals and less common cereals”. This contained an extended review on sorghum and was published in 2002.
A list of publications resulting form the project is given in Table V.

Peer reviewed journals

Duodu, K.G., Taylor, J.R.N., Belton, P.S., & Hamaker, B.R. Factors affecting sorghum protein digestibility. *J. Cereal Sci*. (2003) (in press).

Hugo,L.F., Rooney, L.W., & Taylor, J.R.N. Fermented sorghum as a functional ingredient in composite breads *Cereal Chem.* (2003) (in press).

Pelembe, L.A.M., Erasmus, C. and Taylor, J.R.N. Extrusion cooked sorghum-cowpea porridge. *Lebensm. -Wiss. u. -Technol*. **35** (2002) 120-127

Duodu, K.G., Nunes, A., Delgadillo, I., Parker, M.L., Mills, E.N.C., Belton P.S. and Taylor, J.R.N. effect of grain structure and cooking on sorghum and maize *in vitro* protein digestibility. *J. Cereal Sci.* **35** (2002), 161-174

Taylor, J. & Taylor J.R.N. Alleviation of the adverse effects of cooking on protein digestibility in sorghum through fermentation in traditional African porridges. *Int. J. Food Sci. Technol.* **37** (2002) 129-138

Duodo, K.G., Tang, H., Grant, A. Wellner, N., Belton, P.S. and Taylor, J.R.N. FTIR and solid state nmr spectroscopy of proteins of wet cooked and popped sorghum and Maize. *J. Cereal. Sci.* **33(**2001261-269

Hugo L.F., Rooney L.W., Taylor J.R.N. Malted sorghum as a functional ingredient in composite bread *Cereal. Chem*. **77** (2000) 428-432

Parker, M.L., Grant, A. Rigby, N.M., Belton P.S. and Taylor, J.R.N. effects of popping on the endosperm cell walls of maize and sorghum. *J. Cereal Sci.,* **30** (1999) 209-216

Taylor, J.R.N., Donaldson, A.S. and Dewar J. Improvement in sorghum malt quality through alkaline steeping. *Cereal Foods World*. **43 (**1998) 532

Conference Proceedings

Belton, P.S., Delgadillo, I., Grant, A. and Taylor, J.R.N. NMR and FTIR studies of cereal proteins. In " Spectroscopy of Biological Molecules: Modern Trends", (P. Carmona, R. Navarro and A. Hernanz, eds) Kluwer Academic, Dordrecht, (1997) pp 499-500

Taylor, J.R.N., Dewar, J., Belton,P.S., Delgadillo, I., Hugo, L.F., Pelembe, L.A.M. and Wambugu, S.M. The Problem of poor protein digestibility in sorghum. In "Harnessing Cereal Science and Technology for Sustainable Development" ICC-SA, Pretoria (1997) pp90-91

Delgadillo, I., Taylor, J.R.N., Dewar, J., Belton, P.S., Hugo, L.F., Pelembe, L.A.M. and Wambugu, S.M. Mejoramieneto de la digestibilidad de la proteína del sorgo. In Simposio Internacional: Biotechnolgia en la Industria de Alimentos, (J. Ruales, C. Carpio, J. Bravo and P. Santcruz, eds) Escuela Politécnica Nacional, Ecuador, 2000 (publication date 1999) pp147

Books

Increasing the Utilisation of Sorghum, Buckwheat, Grain Amaranth and Quinoa for Improved Nutrition" -Selected Papers from the ICC Cereals Conference Symposium Challenges in Speciality Crops. Vienna 1998. (P.S. Belton and J.RN. Taylor eds Institute of Food Research, Norwich (1998)

Information Handbook: Sorghum Malting, Sorghum Fermentation, Preparation of Composite Bread and Instant Weaning Food" CSIR, Pretoria (1999)

Manuel Informativo Maltagem e fermentação da Mapira(Sorgo), Preparação de Pão e Papinhas Instantâneas para Bebé na base da Mapira CSIR, Pretoria (1999) Pseudocereals and Less Common Cereals (P.S. Belton and J.R.N. Taylor eds) Springer, Berlin (2002)

Dissertations and theses

L.F. Hugo. 2002. Malted and fermented sorghum as ingredients in composite bread. PhD, University of Pretoria

K.G. Duodu. 2000. Role of grain organisational structure in sorghum protein digestibility. PhD, University of Pretoria

S.M. Wambugu. 1999. Malted and fermented sorghum as functional ingredients in extruded instant weaning porridges. MSc With Distinction, University of Pretoria

A.S. Donaldson. 1999. Steeping and sorghum malting. MSc, University of Pretoria

L.A.M. Pelembe. 1998. Extrusion cooked sorghum-cowpea instant porridge. M Inst Agrar, University of Pretoria
Paulo Alexandre Teixeira Marques 2002.Extracção, Purificação e Caracterização Bioquímica de uma ProteinaseAspártica de Sementes de Sorghum Bicolor (L.) Moench. Master Diss. University of Aveiro,

Carla Alexandra Pina da Cruz Nunes 2002. Contribuição para a caracterização da fracção de prolaminas de duas variedades de sorgo. Master Diss. University of Aveiro,

Table V Publications, dissertations and theses from the project.

Training and Exchanges

Training and exchange formed and important part of the project. One participant from Kenya spent two years working at the University of Pretoria and two participants from Mozambique spent two years at the same institution. A Ghanaian student, funded by a Commonwealth Fellowship, worked on the project in the University of Pretoria and the Institute of Food Research. A student from France, funded under the Leonardo scheme, carried out a research project on polyphenols in sorghum and maize at IFR and students from the University of Aveiro also studied for periods at IFR. Short training visits by the participants at IFR and UA also took place.
Overall the project resulted in the publication of 6 theses and dissertations.

CONCLUSIONS

Overall the project may be judged to have been a success. A strong network was created which has continued to be productive. Members of the group have been heavily involved in the planning this conference and form a part of the team for a second EU funded project on Sorghum. The outcomes in terms of scientific output were considerable, as indicated in Table V and have constituted a significant advance in knowledge about scientific and technological aspects of sorghum. Practical outputs have been in terms of the new products sorghum based bread and weaning food. These have not only been of value of themselves but have acted as demonstrations of possible approaches to other novel products. Training has also been very effective.

An important aspect of the project has been its dissemination strategy. The combination of workshops and information booklets has enables a wide variety of potential users to familiarise themselves with the results and have them presented in manner suitable for utilisation.

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3. Parker, M.L., Grant, A. Rigby, N.M., Belton P.S. and Taylor, J.R.N. Effects of popping on the endosperm cell walls of maize and sorghum. *J. Cereal Sci.,* **30** (1999) 209-216

4. Duodo, K.G., Tang, H., Grant, A. Wellner, N., Belton, P.S. and Taylor, J.R.N. FTIR and solid state nmr spectroscopy of proteins of wet cooked and popped sorghum and maize. *J. Cereal. Sci.* **33(**2001) 261-269

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6. Grant, A., Belton, P.S., Colquhoun, I.J., Parker, M.L., Plijter, J. Shewry, P.R. Tatham, A.S. and Wellner, N. The effects of temperature on the sorption of water by wheat gluten determined using deuterium NMR. *Cereal Chem.* **76 (**1999) 219-226

7. Taylor, J.R.N. and Belton, P.S. Sorghum. In “Pseudocereals and Less Common Cereals” (P.S. Belton and J.R.N Taylor eds) Springer, Berlin (2002) pp 25-91

8. Taylor, J.R.N., Donaldson, A.S. and Dewar J. Improvement in sorghum malt quality through alkaline steeping. *Cereal Foods World*. **43 (**1998) 532

9. For further details see “Information Handbook: Sorghum Malting, Sorghum Fermentation, Preparation of Composite Bread and Instant Weaning Food” CSIR, Pretoria (1999)

10. Hugo L.F., Rooney L.W., Taylor J.R.N. Malted sorghum as a functional ingredient in composite bread *Cereal. Chem*. **77** (2000) 428-432