Swedish University of Agricultural Sciences
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Independent project • 15 hec • First cycle, G2E
Biology • ISSRN SLU-MIKRO-EX - 11/8-SE: 2011:8 • ISSN 1101-8151
Uppsala 2011

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Credits: 15 hec
Level: First cycle, G2E
Course title: Bachelor project
Course code: EX0689
Programme/education: Biology

Place of publication: Uppsala
Year of publication: 2011
Picture Cover: Field in Dschang Cameroon where maize is grown together with green beans. Photo: Sofia Ny
Title of series: no: ISSRN: SLU-MIKRO-EX - 11/8-SE: 2011:8
ISSN: 1101-8151
Online publication: http://stud.epsilon.slu.se

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Abstract

Post-harvest loss is a major problem for farmers in sub-Saharan Africa. Substantial amounts of cereal grain are lost each year due to factors such as exposure to rain, extreme drought, physical damage and invasion of microorganisms. This decreases the availability of food/feed which in turn increases prices and the consumption of unsafe food. Therefore storage systems need to be developed to ensure a safe food supply with low losses.

The project aimed to evaluate the inhibiting effects of *Meyerozyma guilliermondii* against mould growth on maize under local conditions in Cameroon. This was in order to determine if *M. guilliermondii* could be used for biocontrol storage of moist maize under airtight conditions. Previous studies have shown this system, together with the yeast *Wickerhamomyces anomalus* as biocontrol, to be very effective for grain preservation.

Mini silos were inoculated with different moulds and yeasts. *W. anomalus* was used as a control since it has previously been shown to have an inhibiting effect on mould growth.

Furthermore the project aimed to study local post-harvest systems for maize in two different areas of Cameroon. This was done by means of visiting farms and interviewing farmers. The new biocontrol system of moist grain was also explained to the farmers in order to introduce the idea to them and see if they were willing to try a new storage system. A hygiene study of the storages was also done by microbial quantification and calculation of colony forming units (CFU).

No inhibition of mould growth was detected for either *M. guilliermondii* or *W. anomalus*. Several factors such as low moisture content, too much mould and the size and the material of the mini silos made the results questionable. More studies need to be made regarding the biocontrol activity of *M. guilliermondii.*

The storage method and hygiene varied between different farms in the Nforya-Bamenda area. However none of the local storage systems seemed to have better hygienic status for yeasts and moulds. The biocontrol system seemed suitable for introduction in Nforya-Bamenda for several practical and socio-economic reasons. With current storage technology it would be no use increasing yield per hectare for maize in this region since farmers already produce more than they can store. Moreover, the change in maize storage technology could prove essential to improving women and children's health in Cameroon, since many farmers smoke their maize indoors using fire-wood.

Keywords: Cameroon, Post-harvest loss, Biocontrol storage, *Meyerozyma guilliermondii, Wickerhamomyces anomalus*, Local post-harvest systems, hygiene analysis

Abbreviations: CFU: colony forming units. FAO: Food and Agriculture Organization of the United Nations. Y31: *Wickerhamomyces anomalus*. Y51: *Meyerozyma guilliermondii*

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1. Introduction

In February 2011 the FAO food price index rose above the levels of 2008 making food more expensive than ever before (FAO 2011). With an increasing world population the demand for more food has risen to a critical stage. Efforts have so far mainly been focused on increasing area yield through crop breeding programs, increased use of fertilizers, irrigation and other modernizations. However rising food prices and the recent financial crisis have turned more attention towards post-harvest loss.

Post-harvest loss is a major problem for farmers in developing countries. Substantial amounts of cereal grain are lost each year due to factors such as exposure to rain, extreme drought, physical damage and invasion of microorganisms. This decreases the availability of food /feed which in turn increases prices and the consumption of unsafe food (FAO 2009).

In a recent report from FAO, World Bank and UK's Natural Resources Institute it was estimated that sub-Saharan Africa loses 10-20% grain prior to processing. Total losses could meet the minimum food requirements for 48 million people annually. It was also concluded that more research is needed in order to adapt new and existing technologies to local conditions (FAO/World Bank/UK's Natural Resources Institute 2011). Storage systems therefore need to be developed to ensure a safe and reliable food supply.

A way of storing moist grain together with a biocontrol organism is currently studied at the Department of Microbiology at SLU. Previous studies have found that the yeast *Wickerhamomyces anomalus* inhibits mould growth and helps preserve moist grain. It does so without disturbing the population of lactic acid bacteria that are important for fermentation of the grain. This has been proven in several studies with small and large scale silos. This type of storage is effective even in malfunctioning silos with air leakage (Olstorpe, M. et al, 2010a, Druvefors, U. Ä. et al. 2004. Petersson S. et al. 1995). So far studies have only been made under Swedish conditions with cereal grain that is commercially grown here like barley and wheat.

The process is started with inoculation of *W. anomalus* in the beginning of the storage period. In order for the fermentation process to be optimal, high moisture content is preferable. The grain can then be stored airtight in plastic sacks or containers (Olstorpe, M. et al, 2010a).

This system could be suitable for introduction in tropical developing countries like Cameroon for several reasons. It uses less energy compared to heated air drying (Olstorpe, M. et al, 2010a). It is important to keep in mind that most countries in Africa use other ways of drying grain like smoke or sun. In Cameroon most farmers harvest during the heavy rain season in July-September. Consequently, with these methods it takes up to several months to reach moisture contents around 10%, which is recommended to minimize microbial proliferation (Mboge M. G 1995).

It also uses simple storage devices such as plastic containers. This makes the initial investments low in cost.

At least 76% of the population in Cameroon depends on biomass fuels like wood, dung and agricultural residues for their basic energy needs. Open indoor fires are used for cooking, heating and light. The incomplete combustion creates pollutants like carbon monoxide and small particles (2.5-10 µm) that can penetrate deep into lungs. The exact mechanism of how indoor smoke causes illness is yet unknown and more research is needed on the subject. There is, however, strong evidence that indoor air pollution is connected to several respiratory diseases including chronic obstructive pulmonary disease and acute respiratory infection in childhood. The latter is the most important cause of death for children under the age of 5 in developing countries. Women and young children (traditionally carried on the mother's back) suffer from higher exposure levels since women are often responsible for cooking. Studies have been made in order to estimate the impact indoor air pollution has on the global disease burden. It was estimated at just below 4% of the disability adjusted life year loss. This means that the impact indoor air pollution has on health is only exceeded by malnutrition (16%), unsafe water and sanitation (9%) and unsafe sex (4%). This makes indoor air pollution a major global health problem that affects mainly poor people in developing countries and is comparable to the health effects of tobacco. Yet it does not receive as much attention as many other issues which means that efforts are needed to raise public awareness (Bruce N. et al 2000).

In sub-Saharan Africa there are major concerns regarding mycotoxins. These are secondary metabolites produced mainly by different species of *Penicillium*, *Aspergillus*, and *Fusarium*. There is consistent evidence that mycotoxins are present in unacceptable levels in dietary staples but the risks of consuming such food is often overlooked. This is due to several socio-economic factors and often food shortage leaves no other choice but to consume contaminated food or to use it as animal feed. Maize is particularly sensitive to contamination by aflatoxins and fumonisins produced by species of *Aspergillus* and *Fusarium*. Aflatoxin B1 is classified as a group one carcinogen and is associated with liver cancer if consumed daily in small doses. Liver cancer is the third deadliest type of cancer in Africa. There is further evidence that daily intake increases the risk for immune-suppression and impaired growth. Thus efforts are being made to minimize mycotoxin contamination in food/feed around Africa. It has been shown that good agricultural practices can decrease the presence of mycotoxins substantially. This includes proper storage and drying, insect management and sanitation. There is a strong relationship between insect damage and mould/mycotoxin contamination. Insects carry the spores from plant and soil to the grains and cobs. Therefore one can be certain that there is a problem with moulds if the maize has been damaged by insects (Wagacha J.M. et al, 2008).

Since *W. anomalus* has not been isolated from maize storages in Cameroon, alternative biocontrol yeasts need to be evaluated (Olstorpe M. et al unpublished results 2011). It might be that *W. anomalus* is not part of the common flora in Cameroon or that it does not grow well on maize. Another yeast that has been studied for biocontrol purposes is *Meyerozyma guilliermondii*. It has shown some inhibiting activity on mould growth (Druvefors U. Ä. et al. 2004). *M. guilliermondii* was commonly isolated from maize storages in Cameroon, especially after 5 months of storage (Olstorpe M. et al unpublished results 2011).

The purpose of this study was to investigate the biocontrol activity of the yeast *M. guilliermondii* isolated from a maize storage in Cameroon. This was done by setting up biocontrol tests with *M. guilliermondii* and *W. anomalus* as comparison. The experiments were carried out at the University of Dschang in Cameroon. The method of mini silos developed by Petersson, S. et al. 1995 was used. To see what effect the possible biocontrol organisms had on mould growth under local conditions, incubation was done in room temperature. This means that the temperature varied from day to night. Since no pure cultures were present at the department two different mixes of moulds were used, one of them being a contaminated sample consisting of *Aspergillus* spp.

The project also aimed to do a study and a hygiene control of local post-harvest systems. This was done by means of interviewing farmers in the two areas Bamenda and Dschang. Samples were taken and their storage systems were photo documented. The purpose was to introduce the idea of a new storage system and get a general feel whether farmers would be positive towards trying a new way of storing. We also wanted to see if this type of storage was suitable to the location and the needs of the farmers.

This was an important part of the study since new storage systems are notoriously difficult to introduce, which is highlighted in the book “Grain storage techniques” by FAO. Social and economic investigations are necessary to decide if a new storage method could be successful. Farmers are often reluctant to adopt new storage technologies. The farmers’ priorities are not always to reduce post-harvest loss but instead to reduce costs and labor. Previous studies have shown that farmers can tolerate high losses before they are willing to invest in new storage technology. In fact, sometimes technology that increases losses has been implemented because of cuts in costs. The introduction of new storage systems have often been unsuccessful because it has simply been assumed that farmers want to improve their storage, which is not always the case. For these reasons it is important to understand why and how farmers store and what problems they experience before starting to introduce a new system (FAO 1994). In order for the new storage system to be successfully introduced, efforts to establish the project and to build networks need to be made.

2. Methods and Materials

The experimental work of this project was carried out at the Animal Health Laboratory, Department of Animal Production, Faculty of Agriculture and Agricultural sciences, University of Dschang, Cameroon. Therefore certain compromises regarding sterile techniques and other equipment had to be made. When incubation was made in room temperature samples were left on the bench which had outdoor temperature that varied between approximately 10-25 ºC.

2.1 Preparations of maize samples for biocontrol tests

The maize used for this analysis was bought from the central market in Dschang. A triplicate of maize was suspended in peptone water (containing 2g of peptone and 3 drops of tween 80/l), 10x sample weight and left for a few hours in room temperature. The bags containing the maize samples were then shaken vigorously for 2-3min by hand before being serially diluted and spread on different culture substrates.

For media recipes see Appendix 2. All medias were autoclaved to 120°C for 20 min before use (except for VRBG that was boiled for 20 min). However no autoclave tape was available to verify if the procedure were successful. Yeasts were grown on Yeast Peptone Dextrose (YPD) agar, incubated 2 days in room temperature. Moulds were quantified on Dichloran-Glycerol (DG18) agar, incubated 6 days in room temperature. *Enterobacteriaceae* were quantified on Violet Red Bile Glucose (VRBG) agar, incubated 1 day in 30 ºC, using pour plate method. The total number of aerobic bacteria was quantified on Tryptone Glucose Extract Agar (TGEA), incubated for 3 days in 30 ºC, using pour plate method. After incubation mean values of colony forming units (CFU) gram^{-1} grain were calculated.

3kg of maize was treated with 300 ml water per 1000g of maize. Water was boiled for 20 min and then chilled to room temperature before use. The moist maize was left for 6-7 days in refrigerator to allow it to obtain the right moisture content. The moisture content of the dry and moist maize was determined by drying in 80ºC for 72h.

2.2 Inoculation of maize

A loop of overnight cultures from the yeasts *W. anomalus* strain J121(coded Y31) or *M. guilliermondii* (coded Y51) was suspended in peptone water and vortexed vigorously for a few minutes. Both yeast cultures were stored at -70ºC by the Department of Microbiology at SLU prior to usage. The same treatment was used on sporulating colonies of *Aspergillus* spp and an unknown mould species isolated from corn. To determine the concentration of the suspensions a Bürcker cell counting chamber was used. The prepared maize was divided and inoculated separately with *W. anomalus* and *M. guilliermondii* to a concentration of 10^5 CFU/g and the moulds to a concentration of 10^3CFU/g. This was done by adding drops of the suspension while mixing the maize by hand. The volumes added differed slightly depending on the concentration of the spore/yeast solution. From each inoculation 10g samples was shaken and diluted serially with peptone water. The number of CFU/g of yeasts and moulds were then determined by cultivating on YPD and DG18 as described earlier.

2.3 Inoculation of mini silos

As mini silos plastic tubes with hard cotton lids punctured with a syringe were used, see Figure 1. 5 different triplicates were prepared; 1) Control, containing only the moist maize, 2) Yeast, containing maize inoculated with either *W. anomalus* or *M. guilliermondii*, 3) Moulds, containing only maize inoculated with either *Aspergillus* or the unknown mould, 4) Mix of *W. anomalus* with *Aspergillus* or unknown mould, 5) Mix of *M. guilliermondii* with *Aspergillus* or unknown mould. The mixture was made by adding every other maize grain from the yeast and the mould. The mini silos were incubated in dark in room temperature for 5 ½ weeks. The content of the mini silos was then added to stomacher bags and suspended (10x its weight) with peptone water. The samples were shaken and serially diluted. To quantify the number of yeasts and moulds the samples were grown on YPD and DG18 as described above.

2.4 Collecting samples and interviewing farmers

Samples and interviews were done in two different areas of north-west Cameroon in April and May 2011. From the village of Nforya, close to Bamenda, located 1240 m above sea level at approximately latitude 6° North and longitude 10° East 10 farms were visited. In the city of Dschang, located 1380 m above sea level at approximately latitude 5° North and longitude 10° East, 7 farmers were interviewed. At each farm three samples from the maize storage were collected. Since farms differed in size and post-harvest systems the samples looked very different. Some were empty cobs, sheaves, dried spices and other debris found on the attic floor. Some of the storages still had maize left in which case both the grains and sheaves were used. All samples collected were taken from three different places in the storage space.

Figure 1. Inoculated mini silo

The farmers were also interviewed about their current storage and harvesting methods after our purpose and the new storage system had been explained. The questions asked were the following:

1) How do you harvest and currently store your maize?
2) For how long do you store the maize?
3) What do you use the maize for?
4) What are the biggest problems with your current storage system?
5) Would you be open to try a new storage system?
6) Which local storage system would you prefer and why if you had the financial means?

2.5 Microbial quantification of local maize storages

5g from each sample, from the 10 farms, was suspended in 45 ml peptone water and shaken vigorously for 2-3 min. The samples were then serially diluted with peptone water and spread on different culture substrates. The plating volume used was 0.1 ml.

Yeasts were grown on Yeast Peptone Dextrose (YPD) agar, incubated 2 days in room temperature. Moulds were quantified on Dichloran-Glycerol (DG18) agar, incubated 5 days in 25 ºC. *Enterobacteriaceae* were quantified on Violet Red Bile Glucose (VRBG) agar, incubated 1 day in 30 ºC, using pour plate method. The total number of aerobic bacteria was quantified on Tryptone Glucose Extract Agar (TGEA), incubated for 3 days in 30 ºC, using pour plate method. After incubation mean values of colony forming units (CFU) gram^{-1} grain were calculated.

3. Results

3.1 Initial hygiene study and inoculation concentrations for biocontrol tests

The results from the hygiene analysis of the number of yeasts, total aerobic bacteria, *Enterobacteriaceae* and moulds made from the maize used for the biocontrol tests are shown in Table 1. No *Enterobacteriaceae* or yeasts were detected. The YPD plates, used to quantify yeasts, were overgrown with moulds.

Table 1. Values from microbial quantification from maize used for biocontrol in $\log_{10}$ units CFU/g

Variable	Biocontrol maize	STDEV*
Enterobacteriaceae	Bd*	-
Yeast	Bd	-
TAB*	3.40	0.099
Mould	4.04	0.671

*TAB: Total Aerobic Bacteria, *STDEV: Standard deviation.* Bd: Below detection

The result from the quantification of yeasts and moulds, on YPD and DG18, of the inoculated maize are shown in Table 2. The standard deviations were calculated from the $\log_{10}$ values. A notable result was that the numbers of moulds were lower after inoculation of the maize with 10^3 spores/g maize from different moulds then from the initial hygiene analysis, see Table 1 and 2. The inoculations of yeast were close to 10^5 CFU/g maize, which was the intended concentration.

The moisture content of the untreated maize was 5.6%. After it was soaked for 7 days the moisture content was 19%.

Table 2. Values from microbial quantification from the inoculated maize used for biocontrol in $\log_{10}$ units CFU/g and in CFU/g

Variable	IM*$\log_{10}$ CFU/g	IM CFU/g	STDEV*
Aspergillus mix	3.97	$9.4*10^3$	0.170
Mould mix	3.87	$7.4*10^3$	0.194
Y31	4.90	$7.9*10^4$	0.105
Y51	5.21	$1.6*10^5$	0.191

*IM: Inoculated maize. *STDEV: Standard deviation

3.2 Microbial quantification from biocontrol tests

In Table 3 the results from the hygiene control are presented. These samples were not inoculated with anything; they only contained the moist maize. Moulds had grown more than 4 log_{10} CFU/g maize during the 5 ½ weeks incubation. Yeasts remained at 0 CFU/g as in the first hygiene analysis, Table 1. *Enterobacteriaceae* had, surprisingly, grown from below detection limit to 6.63 log_{10} CFU/g. No results from total aerobic bacteria could be determined because of contamination.

Table 3. Values from microbial quantification from maize used for biocontrol in log_{10} units CFU/g

Variable	Biocontrol maize	STDEV*
Enterobacteriaceae	6.63	0.303
Yeast	Bd*	-
Mould	8.45	0.083

*STDEV: Standard Deviation. *Bd: Below detection

In Table 4 values from the quantification of moulds and yeasts after 5 ½weeks incubation are presented. All values for moulds were similar ranging approximately from 8-9 log_{10} CFU/g. Yeasts had grown from the inoculated values around 5, see Table 2, to between 6-9 log_{10} CFU/g. There were no values that showed any indication of inhibition in the biocontrol tests.

Table 4. Values from microbial quantification from the biocontrol incubated for 5 ½ weeks in log_{10} units CFU/g

Variable	Moulds	STDEV*	Yeasts	STDEV*
Asp*	8.11	0.040	-	-
M*	8.14	0.210	-	-
Y31*	-	-	6.83	0.347
Y51*	-	-	7.10	0.270
Asp/Y31*	8.28	0.102	6.61	0.270
Asp/Y51*	8.25	0.155	6.97	0.164
M/Y31*	8.29	0.137	9.68	2.016
M/Y51*	9.29	0.116	9.05	0.889

*STDEV: Standard Deviation. Y31: *W. anomalus*. Y51: *M. guilliermondii.* Asp: Control incubated with *Aspergillus*. M: Control incubated with different moulds. Y31: Control incubated with only yeast coded Y31. Y51: Control incubated with only yeast coded Y51. Asp/Y31: Biocontrol incubated with *Aspergillus* and yeast Y31. Asp/Y51: Biocontrol incubated with *Aspergillus* and yeast Y51. M/Y31: Biocontrol incubated with different moulds and yeast Y31. M/Y51: Biocontrol incubated with different moulds and yeast Y51

In Figure 2 the values from Table 3 and 4 have been visualized. It is clear that no inhibition of mould growth was observed in any of the cases

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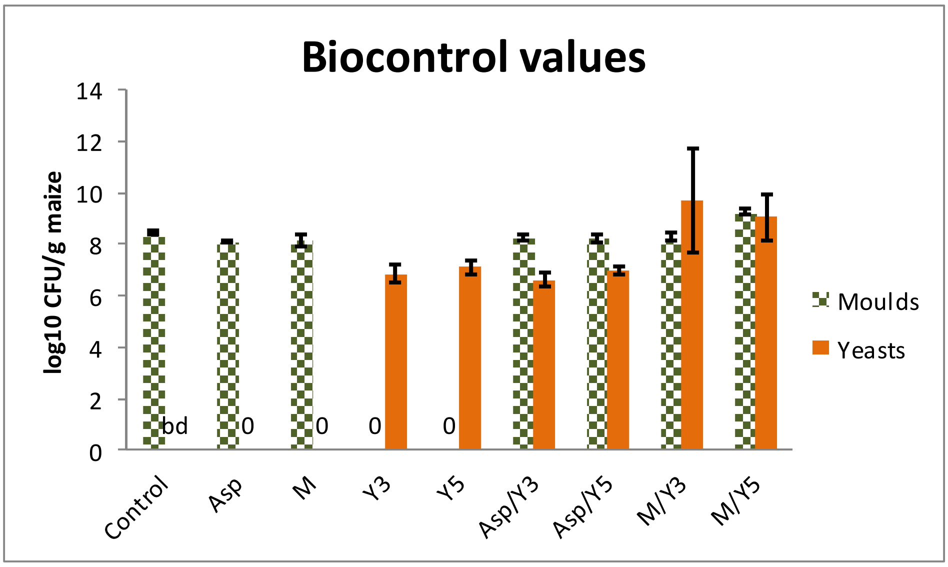

Figure 2. Quantification of moulds and yeasts after incubation for 5 ½ week.

3.3 Local post-harvest systems in Nforya-Bamenda

For all answers from farmers see Appendix 1. In the village of Nforya several ways of storing maize are currently used. It is a rural area about 40 min drive from Bamenda city center. Farms usually consist of several buildings with large outdoor spaces. The maize is most commonly used for food and feed since many farmers also have animals.

The most common harvest period is during the rainy season in July to September. The maize is then dried by smoking in the combined kitchen storage facility. Two different ways of storing are commonly used, hanging or placing in the attic, see Figure 3, 4 and 7. When the maize is hung the cob with its sheaves are tied up and hung from the kitchen ceiling. This is labour intensive and often means that the newly harvested maize stays on the ground for a couple of days before it is hung. The other method is to place the unpeeled cobs on the attic floor above the stove. When the maize is in the attic it requires turning about every other day in order for the drying process to be effective if several layers of maize are added. A fire is then kept 24h a day for 1-2 months. Since the same facility is used for both types of storage they are often combined since farmers often find it difficult to fit all their harvest into one system. After the maize is dry it is often peeled from the cob and put in bags or containers with insecticide.

A different method is to use so called cribs, see Figures 5 and 6. In this system the maize is peeled of its sheaves and put straight into the crib where the open air dries it. This system is less common but when farmers were asked which system they would prefer most answered cribs. The general view seems to be that it is better because of fewer attacks from an insect called weevil and because it is less labour intensive.

Figure 4. Local kitchen/storage where maize is hung from the ceiling.

Figure 3. Empty storage attic where maize is stored on the floor.

Figure 5. A local crib used for storing maize.

Figure 6. Peeled maize stored in local crib.

3.4 Local post-harvest systems in Dschang

Dschang is an urban area which means that most farmers have their storages and fields at different places. Maize farming is done by women and is often not the main income but a way to supply the family with food and some extra money. Houses are located close to each other and the outdoor spaces are often small.

The most common harvest period is during the heavy rain season in July to September. Two ways of storing maize were used by the farmers interviewed, drying by sun or smoke, see

Figure 7. Since it is an urban area the maize needs to be transported from field to the house for storing. This is a big problem for many farmers since most do not have cars. The maize is then put in the space between the ceiling and the tin roof (looks similar to Figure 3) to allow the sun to dry it. Some farmers only store their maize above the kitchen in which case it gets smoked. Unlike the farmers in Nforya the maize is only stocked in one layer which means that it requires less turning. The maize commonly stays in the attic in 2-3 months before it is peeled, commonly sprayed with insecticide and put in containers.

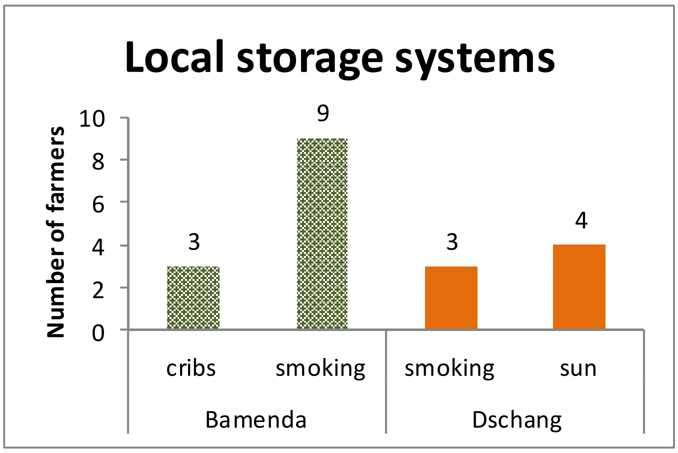

Figure 7. The distribution of storage methods in Bamenda and Dschang shown in numbers of the interviewed farmers

3.5 Storage problems and general opinions regarding the biocontrol storage system

In Figure 8 the most common problems according to the farmers are presented, for interviews see Appendix 1. In both regions farmers have huge problems with insect control. An insect called weevil seems to be causing the biggest problems since it attacks the smoked maize while it is in the barn (hanging or in the attic). Many farmers seem to prefer the crib system because of fewer problems with weevils. However some farmers with the crib system have problems with other types of insects. It is not very common that the farmers experience that they have a problem with moulds in any of the regions. However there are exceptions. One farmer in Dschang (D8) estimated that she often lost half of her harvest due to moulds.

Most farmers that smoke their maize had a problem with getting enough fire-wood that according to farmers has increased a lot in price during the last years. All farmers interviewed in both areas used wood as energy source. Another interesting result was that many farmers in Nforya often harvest more maize then they have place to store. In Dschang this problem was much smaller.

Another result that differed between the two areas was what the maize was used for. In Nforya it was used for food and feed since farmers often had animals. In Dschang maize was mainly used as food and for selling as food in the market.

Furthermore storage time differed a lot between farmers. Many said that they keep the maize from one harvest to the next which means for one year. However many of these farmers' storages were empty. How long a farmer stores the maize seems to be more connected with how fast they consume it and little with the quality of the maize.

In Nforya all farmers were positive and willing to try the biocontrol storage system. In Dschang farmers had more reservations and were much more concerned. Many wanted to see the product and how the storage worked before trying themselves. Concerns about how to get the material, yeast and how to peel the fresh cobs were expressed.

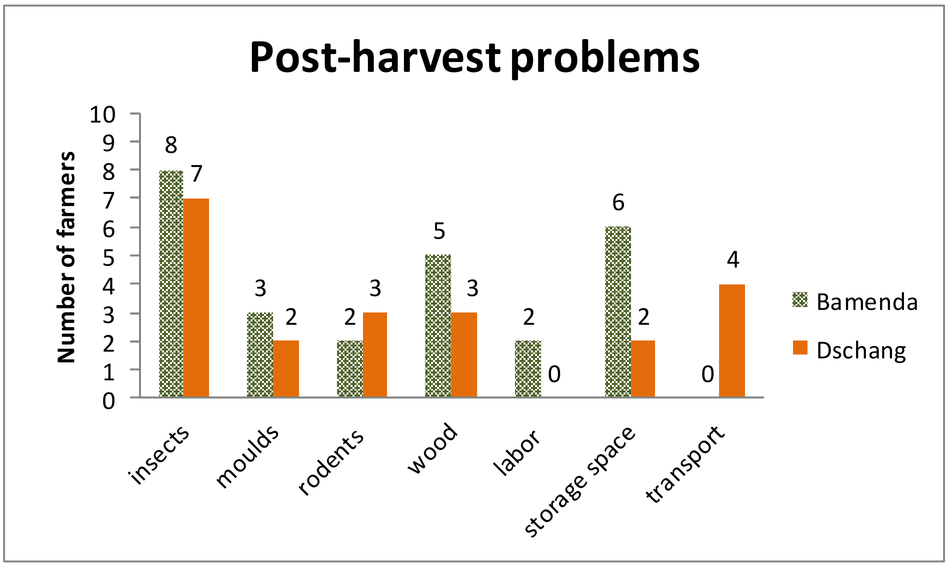

Figure 8. Diagram showing the most common post-harvest problems according to the farmers

3.6 Hygiene study of local storages in Bamenada

In Table 5 the results from the hygiene study from the storages are presented. The farmers are represented by code B1-B10. The values from yeast were calculated from DG18 after 2 days incubation in room temperature since the YPD plates were too contaminated with moulds to calculate. The TGEA plates used to quantify total aerobic bacteria were contaminated with both moulds and larvae from flies which made it hard to distinguish what were bacteria so no certain result can be presented. The quantification of *Enterobacteriaceae* was done twice but with no certain result. There were a lot of microorganisms growing on the plates but non that could be distinguished certainly as *Enterobacteriaceae* judging from the shape and colour. It is obvious that the hygiene status (regarding yeasts and moulds) of the storages were very different between farms. Some of the standard deviations were quite high. An interesting result was that the farms that had high numbers of yeasts also had high numbers of moulds (and vice versa), see Table 1 B5, B6 and B9. This suggests that these storages were more favourable for the proliferation of microorganisms. Another interesting result was that these farms B5, B6 and B9 all used different storage methods, see Appendix 1. Farm B5 tied up and smoked the maize, B6 put the maize straight in the attic and smoked it while B9 used the crib system.

Table 5. Microbial quantification in log10 units CFU/g from empty storages in Nforya

Farmers	Yeast	STDEV*	Mould	STDEV	Storage method
B1	6.15	1.86	4.67	0.538	Cribs
B2	4.90	2.69	3.29	0.244	Smoke/attic
B3	2.96	0.20	3.65	0.340	Smoke/hanging
B4	3.75	2.44	3.27	0.324	Smoke/hanging

B5	8.38	1.08	7.24	0.783	Smoke/hanging
B6	8.15	0.85	7.01	1.86	Smoke/attic
B7	5.04	0.58	3.09	0.08	Smoke/attic
B8	4.43	2.54	3.71	0.75	Smoke/hanging
B9	8.07	0.60	7.43	1.02	Cribs
B10	4.27	0.49	4.20	0.66	Cribs

*STDEV: Standard deviation

In Table 6 and Figure 9 and the mean CFU values from yeasts and moulds grouped according to storage methods are presented. Some differences can be observed although none have been statistically proven as significant. Smoking/hanging as storage method seem to have lower numbers of yeasts than the other two methods. The crib system seems to have a higher numbers of moulds.

Table 6. Mean $\log_{10}$ CFU/g sample values for the quantification of yeasts and moulds in relation to different storage methods

Variable	Yeasts	STDEV*	Moulds	STEDV*
Crib	6.16	1.900	5.43	1.745
Smoking /attic	6.03	1.837	4.46	2.208
Smoking/hanging	4.88	2.410	4.46	1.859

*STDEV: Standard deviation

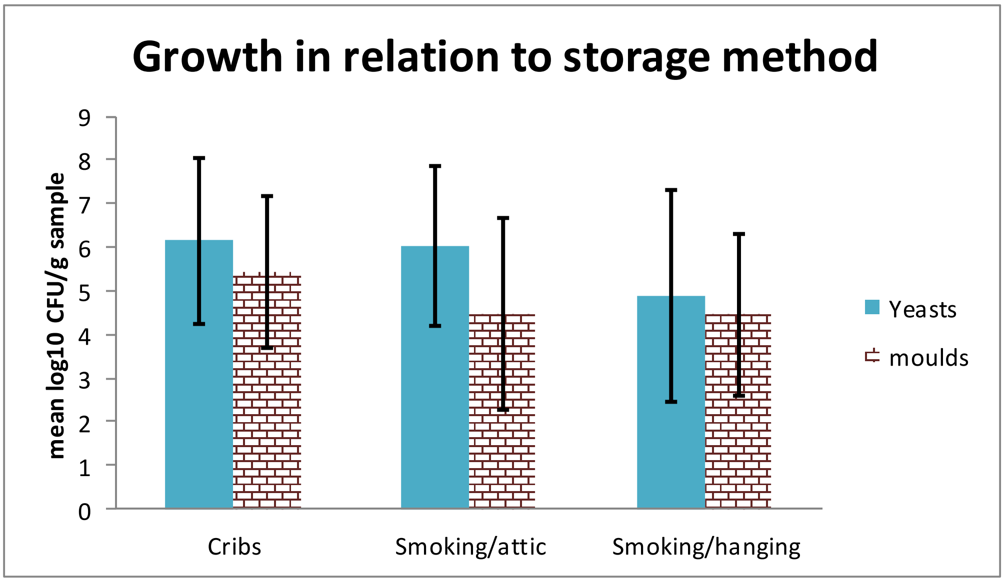

Figure 9. The mean $\log_{10}$ CFU/g sample values for the quantification of yeasts and moulds in relation to different storage systems.

4. Discussion

Wickerhamomyces anomalus has previously been shown to have an inhibiting effect on the growth of moulds in several experiments (e.g Olstorpe, M. et al, 2010, Druvefors U. Ä. et al. 2004, Petersson S. et al, 1995). This makes it suitable to be used as a biocontrol organism in airtight storage of moist cereal grain (Olstorpe, M. et al, 2010). This study aimed to evaluate the biocontrol ability against mould growth of the yeast *Meyerozyma guilliermondii* isolated from local maize storages in Cameroon. *W. anomalus* was used as a comparison to study the difference in inhibition.

Furthermore a study of local post-harvest systems for maize was done in the two different regions Nforya-Bamenda and Dschang, in Cameroon. A hygiene study of empty storages in Nforya-Bamenda was also performed.

None of the yeasts tested showed any signs of inhibiting mould growth. This may have several reasons. First, the concentration of mould was extremely high since the maize already contained moulds at the beginning. After inoculation the maize should have contained around 10^4 CFU/g.

Second, it was not possible to reach the desired moisture content of 30%. This meant that the maize was quite hard and dry which could have affected the yeast proliferation. Moreover the caps used to seal the mini silos were made of hard cotton which probably meant that they absorbed some of the water from the maize.

Third, the plastic tubes used as mini silos were very narrow. Since the maize grains are quite big it was only possible to fit one grain on top of the other, see Figure 1. This could have affected the growth of both moulds and yeasts.

It is also possible that the inhibiting effect of *W. anomalus* that has been observed in other studies does not work so well under the conditions in Cameroon. The strain J121 was originally isolated from a Swedish farm (Petersson S. et al 1995) which means that it is adapted to Swedish conditions. However more studies need to be made on maize before drawing such a conclusion. More studies are also needed on *M. guilliermondii* to determine if it has a future as biocontrol organism on cereals like maize.

Another factor that could be important was that the maize used for the biocontrol tests probably contained too low numbers of lactic acid bacteria (LAB), since it was bought at the end of a storage period. Previous studies on maize stored for more than 5 months in Cameroon showed that the population of LAB was small and homogenous (Olstorpe, M. et. al. 2011 Unpublished results). If no LAB were present there was no fermentation and thus no production of lactic acid. This would be bad for the preservation of the grain, among other things because of the favorable environment for mould growth.

There are big differences between the two areas regarding farmers' attitude towards trying a new storage system. Farmers in Dschang are much more concerned about the system. This could be due to several reasons. The maize is mainly used for food and selling which makes farmers more suspicious towards the end product. Another important reason seem to be that they do not have the same surplus of harvest and thus not the same problem with storage space. This might also be related to that Dschang is an urban area and plots could therefore be smaller in size. Another interesting observation is that farming in mainly done by women in Dschang. The Dschang area has suffered from the close down of many coffee plantations leaving many men unemployed. This means that the women have a large responsibility in bringing in food for their families. This could be a contributing fact in why they are not as willing to risk their harvest. However many of the concerns farmers expressed where of practical things like where the yeast can be bought and where to get the containers. Although these matters might seem small it is good to keep in mind that the infrastructure and political system in Cameroon is in a developing stage. Also most agriculture is done in small scale. This means that systems to grow and distribute cultures of yeasts, and possibly LAB, to many small farms needs to be developed.

In Nforya-Bamenda farms are generally bigger and more animals are kept. Many farmers seem to regard the new storage as an extra system to fit their surplus. All farmers were willing to try the new system and many seemed excited about the idea. They could keep their old systems and just add the new one. In the beginning they could use the maize as animal feed until they get better acquainted with the product. This makes Nforya a better place than Dschang to start introducing the system. There also seem to be a stronger network between the farmers in Nforya. If a few key farmers could be chosen to try the system, and they think it is good, it should be easy to get the others to try it as well. Another important factor is that the farmers in Nforya speak English which makes direct communication possible, which should not be underestimated.

The alternative storage method using *W. anomalus* as a biocontrol organism seems to be suitable for introduction in the Bamenda area for several reasons. Even small plastic silos can fit a substantial amount, if the grains are peeled of the cobs prior to storing. Since storage space is a problem this is desirable. However the process of peeling fresh maize has to be further investigated. Some farmers expressed concerns regarding this. With current storage technology it would be no use increasing yield per hectare by introducing high yielding varieties of maize. Even now farmers are producing more than they can store.

At the moment most farmers conserve maize by smoking. This creates a big demand for fire-wood especially during and a few months after the harvest. In urban and heavily populated rural areas prices and the availability of wood are a rising problem according to farmers. Smoking also creates high levels of indoor air pollution especially during the months when the fires are kept 24h a day. This makes the storing of maize strongly connected to improving women and children’s health in Cameroon. In order for farmers to switch to a cleaner type of energy like gas the storage of maize needs to be changed. It would be financially impossible for farmers to invest in a gas stove and then still have to pay for fire-wood to smoke the maize.

New storage systems are notoriously difficult to introduce (FAO 1994). However, as presented in this report, there are many indications that the farmers in Bamenda are ready for a new system. Their current storage systems are very labour intensive and space demanding. The investments that need to be made in order to adapt the new storage seems manageable considering that other costs, like fire-wood, would be greatly reduced.

The results from the hygiene study of the Nforya-Bamenda showed big differences in presence of moulds and yeasts. Some of these values had high standard deviations. However this was not unexpected since samples were taken from different parts of the storages. No certain conclusions can be made regarding which storage method that has the best hygienic status. The standard deviations were high when mean CFU according to storage system were calculated, Table 6. However the crib system, which was preferred by most farmers, showed no signs in having a better hygiene then the others. One of the reasons to why it was preferred was because of fewer attacks form weevils. Since there is a strong connection between mold contamination and insect damage this result suggests that other insect or larger animals replace the attacks otherwise made by weevils.

5. Conclusions

No certain conclusions regarding the biocontrol activity of *M. guilliermondii* can be drawn. Experiments with fresh maize together with *W. anomalus* and *M. guilliermondii* need to be made. The fresh maize should have a higher moist content and also have a natural occurring population of LAB. They hygienic status of storages in Nforya-Bamenda differs. However there are no indications that any of the present systems are better from a hygienic perspective.

The system of storing moist grain in air tight storage with a biocontrol organism seems suitable for the needs of farmers in the Nforya-Bamenda region. The amount of harvest, what they use their maize for and several socio-economic factors seems to favor this system. The storage of maize needs to be solved to bring a higher standard of living for farmers, and improve women and children’s health.

More investigations regarding practical matters like how to ferment and distribute the yeast needs to be made.

6. Acknowledgements

I want to thank my supervisors Aziwo Niba and Matilda Olstorpe for assistance and support. I am also thankful l for all the help from the personnel at the Animal Health Laboratory, Department of Animal Production at the University of Dschang. A special thank you also goes to all farmers in Nforya-Bamenda: Shu Martin Awaboh, Mankaa Veronica of Nforya, Neba Andrew Chitu, Dorothy Awah Tacheh, Peter Ajuni, Nimang Pims Shu, Daniel Che, Neba John Fuh, Che Chrisautus Che, Lilian Nasi and Dschang: Marié Kinkeu, Delphine Teumo, Pauline and Marie, Suzanne Nguofack, Fransisca Nzomo, Madame Noumedem, Therese Kenfack. I am also grateful for the scholarship form SIDA that made this project possible.

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8. Appendix

1. Interviews with farmers in Nforya-Bamenda and Dschang

Bamenda
B1 Shu Martin Awaboh (Head farmer)

How do you harvest and currently store your maize?
Harvest in July and October, the crib storage we looked at had been harvested in October.The late harvest: Spray a lot of herbicide then bend the stalks of the maize. The maize lie on the ground like that for about a month, getting partially dry. Then they peel it and put it into cribs which is a very airy storage. Having a lot of cats around helps against rodents.

How long can you store your maize?
The late harvest you can store for about 6-7 months.

What do you use the maize for?
The late harvest mostly gets grinded into flour and then used for feed and food.

What are the biggest problems with your current storage system?
Moulds are a big problem and in the early harvest drying the maize to get good seeds for next year is very difficult. No space problem.

Would you be open to try a new storage system?
Yes, especially since I soon want to start having cattle they need a lot of food.

B2 Mankaa Veronica of Nforya

How do you harvest and currently store your maize?
Only early harvest. The harvest goes straight into the barn and smoked everyday for three months (until dry). The maize is turned twice a week to keep from getting mouldy.

How long can you store your maize?
Three months

What do you use the maize for?
Food for the family and feed for poultry plus selling some.

What are the biggest problems with your current storage system?
Weevils. Space is short she puts many layers and turn often.

What current storage system do you think is best?
Cribs

Would you be open to try a new storage system?
Yes

B3 Neba Andrew chitu (Sample from late harvest)

How do you harvest and currently store your maize?

Harvest twice. Early harvest: Maize gets taken from the field hanged over a fire. The maize can lie on the ground for two days before being hanged. The maize gets heavily smoked for about two months then it gets peeled and put into bags were it can lie for another four months. Early harvest is bigger.

Late harvest: The field is sprayed with a herbicide before the maize gets bent and dries for about a month in the field before being hanged over a fire and smoked until dry. The late harvest maize is not smoked as heavily and gets dry a lot faster (in less than a month). It is then peeled and put into plastic containers with lids. Also finds it easier to sell the late harvest because of the market demand.

How long can you store your maize?

Early harvest as long as it is dry about 6 months, late harvest up to a year in the plastic containers.

What do you use the maize for?

Food and feed for poultry and pigs.

What are the biggest problems with your current storage system?

It is labor-intensive, you need a lot of firewood which is expensive. During the early harvest it takes a long time to tie up the maize and some get spoiled while lying on the ground. General problems in the village according to this farmer: Not enough knowledge of when to harvest to get the least problems. Only 10 % of the entire village harvest twice a year. Space problem.

What current storage system do you think is best?

Cribs because it is less labor-intensive and you get a good product.

Would you be open to try a new storage system?

Yes.

B4 Dorothy Awah Tacheh

How do you harvest and currently store your maize?

Harvest once. Tie up the maize over a fire and smoke it for two months then it gets taken down and peeled, sprayed with insecticide. The insecticide works for three months before you need to spray on more. Before eating the maize gets washed, dried and grinded. If the harvest is big the maize also goes up in the barn and has to be turned twice a week.

How long can you store your maize?

Three months in bags, but for longer if you put on more pesticides?

What do you use the maize for?
Food and feed.

What are the biggest problems with your current storage system?
It needs a lot of firewood which is expensive and hard to get by. Weevils and moulds. Sometimes space problems.

What current storage system do you think is best?
The cribsystem because you don't need firewood and it is less labor-intensive.

Would you be open to try a new storage system?
Yes

B5 Peter Ajuni

How do you harvest and currently store your maize?
Harvest twice. Early harvest: The maize gets tied up over a fire and smoked until dry, about two months before it gets peeled and sprayed with pesticide before being put in bags. The maize can lie on the ground for a couple of days before getting tied up. Before eating the maize needs to be washed and dried. Early harvest is bigger. Samples from early harvest.

Late harvest: The maize gets bent and dries in the field for about a month before getting peeled, sprayed with pesticide and put into bags.

How long can you store your maize?
The pesticide lasts for three months so as long as you apply new pesticide every third month you can store it for a long time.

What do you use the maize for?
Food and feed.

What are the biggest problems with your current storage system?
Weevils, getting good quality firewood. Having to use pesticide and then wash before eating. People don't know how to manage late harvest. Space problem.

What current storage system do you think is best?

Would you be open to try a new storage system?
Yes

B6 Lilian Nasi

How do you harvest and currently store your maize?
Harvest once. The maize gets put in the barn straight away, some gets tied up. It is smoked as long as it is in storage. Before being tied up some of the maize might lie on the ground for a day. No pesticides used.

How long can you store your maize?
Up to six months.

What do you use the maize for?
Food and feed.

What are the biggest problems with your current storage system?
Weevils.

What current storage system do you think is best?
Cribs because it is less labor-intensive.

Would you be open to try a new storage system?
Yes.

B7 Che Chrisautus Che

How do you harvest and currently store your maize?
Harvest twice. Early harvest: Maize gets put in the barn straight away and smoked until dry which takes about 3-4 months. The maize gets turned twice a week. When dry it is peeled sprayed with insecticide and put into bags. Early harvest is the biggest. Late harvest: Maize is bent and dried in field for about a month, peeled, sprayed with insecticide and then put into bags.

How long can you store your maize?

What do you use the maize for?
Food and feed.

What are the biggest problems with your current storage system?
Insects and moulds. Not enough space for all the maize, needs a lot of wood for early harvest.

What current storage system do you think is best?
Cribs because it is easier to get rid of weevils, you can just spray it straight away.

Would you be open to try a new storage system?
Yes

B8 Neba John Fuh

How do you harvest and currently store your maize?

Harvest once. The maize gets tied up and smoked until dry which takes at least three months. Before being tied it can lie on the ground for two days at the most. If the harvest is big some of the maize will be taken down sprayed with insecticide and kept in bags.

How long can you store your maize?

For a year as long as it is hanging over smoke.

What do you use the maize for?

Food, feed and wine.

What are the biggest problems with your current storage system?

Insects, that it is labor-intensive. Not enough storage room.

What current storage system do you think is best?

Smoking because then you don’t get any rodents and you have a lot more control over the maize than if it was in a crib.

Would you be open to try a new storage system?

Yes.

B9 Daniel Che

How do you harvest and currently store your maize?

Harvest once or twice depending on how the first harvest went. Early harvest: Gets peeled and put into cribs to dry which takes about two to three months. Then it gets grinded and kept in sealed containers away from insects. The cribs are washed and sprayed with insecticide before new maize is put in them. If there is not enough space in the cribs some maize is put in the barn and smoked. The first harvest is bigger.

How long can you store your maize?

For a long time when it is made into a flour. In cribs for about six months.

What do you use the maize for?

Food and feed.

What are the biggest problems with your current storage system?

Rodents can get in to the cribs, weevils when the maize is dried. Not enough storage room.

What current storage system do you think is best?

The cribs especially if you have a lot of maize since this system is less labor-intensive.

Would you be open to try a new storage system?
Yes

B10 Nimang Pims Shu

How do you harvest and currently store your maize?
Harvest once. The maize is bent in the field and left to dry for about 1 ½ month then it is put into cribs. Some is also put in the barn and smoked.

How long can you store your maize?

What do you use the maize for?
Food and feed.

What are the biggest problems with your current storage system?
Not having enough storage room.

In the cribs: termites eat the legs, rodents, stemborers??

In the barn: Weevils, finding good firewood, more work.

What current storage system do you think is best?
Prefer cribs over smoking in barn because of fewer weevils. Wants another system where you have fire in sealed pipes so the maize gets dry without getting any smoke.

Would you be open to try a new storage system?
Yes

Dschang
We used a translator since none of the farmers spoke English.

D1 Therese Kenfack

How do you harvest and currently store your maize?
Harvest once. Early harvest, puts it straight in the barn (one layer) and smokes it until dry (up to three months) before peeling it and putting it into plastic containers with insecticide. While in the barn the maize gets turned once every two weeks.

How long can you store your maize?
Until it is finished, next harvest which means a year.

What do you use the maize for?
Food and sell some of it to buy fertilizer for next years harvest.

What are the biggest problems with your current storage system?
Transporting the maize, wood, weevils if the maize has dried and not been put in plastic containers straight away. No space problem.

What current storage system do you think is best?
The one I have because I would not be able to fit cribs anywhere.

Would you be open to try a new storage system?
No because the seeds would be destroyed so she thinks it will be difficult to sell.

D2 Madame Noumedem

This woman was our contact person in Dschang. She arranged the meetings with the other farmers. She said that only women farm maize in the area. When asked why and what the men did she explained that men used to work in coffie plantations. Then the market price for coffie dropped and the plantations closed down. This left many men unemployed a few years back.

How do you harvest and currently store your maize?
Harvest once. Early harvest, the maize gets tied up and smoked for three months, until dry. Before being tied, the maize might lie on the ground for a maximum of two days. When dry the maize is peeled and put into plastic containers with insecticide.

If the harvest is big some will be left in the field to dry by itself.

How long can you store your maize?
For a year (until next harvest season).

What do you use the maize for?
Food for the family, if some maize were left and got spoiled in the field she sometimes use it as feed.

What are the biggest problems with your current storage system?
Insects, wood, if you don’t buy enough wood straight after harvest the maize will easily rot. Space, she has to leave it in the field if she can’t fit at home where she ties it up.

What current storage system do you think is best?
Sometimes leaving the maize in the field to dry by itself is best and then peel it and put it in plastic containers. She can’t have cribs because of lack of space at her house. If she had cribs close to the field someone could easily steel the maize.

Would you be open to try a new storage system?
She would be open to try it if we can show her that it is working first.

D3 Fransisca Nzomo

How do you harvest and currently store your maize?

Harvest once. Early harvest, the maize is put straight on the inner roof of the house, one layer of maize. It is then sundried by the heat from the outer roof until dry which takes about three months. The maize is then peeled and put into plastic containers with insecticide. The maize is never turned.

How long can you store your maize?

Up to next harvest, a year, until nothing is left.

What do you use the maize for?

Food for the family, some gets sold and some is used as feed for poultry.

What are the biggest problems with your current storage system?

Rats and weevils because she does not smoke it. No problem with space.

What current storage system do you think is best?

Her own system because it doesn't require any wood.

Would you be open to try a new storage system?

Yes if she can see it that it works she is open to try it.

D4 Suzanne Nguofack

How do you harvest and currently store your maize?

Harvest once. Early harvest, the maize is put straight on the inner roof of the house, one layer of maize. It is then sundried by the heat from the outer roof until dry which takes about three months. The maize is then peeled and put into plastic containers but without insecticide. The maize is never turned.

How long can you store your maize?

Until it is finished which means up to a year.

What do you use the maize for?

Food for the family the maize that has gotten spoiled she sells as feed.

What are the biggest problems with your current storage system?

Transportation from the field to the house. Rats is a huge problem they can destroy up to half of the harvest and the cats can't keep up with killing them all. Weevils, she doesn't have a problem with mould. No space problem.

What current storage system do you think is best?
Her own storage system since the maize is clean on the outside when you peel it and the system doesn't require any wood.

Would you be open to try a new storage system?
She has concerns about how healthy it would be to spray yeast on the maize and also about the costs of buying suitable containers and how well it will keep if she opens the container a lot. If we can show her that the new system works well she would be willing to try it.

D5 Marie

How do you harvest and currently store your maize?
Harvest three times, early; July/August, late; December. The third harvest which is in February is planted in another field, a wetland since the maize grows during the dry season. Same procedure every harvest. The maize is put straight into the barn (inner roof) and gets smoked/sun-dried. There is only one layer of maize and it gets turned twice every week until dry which takes about two months. The maize is then peeled and put into plastic containers with insecticide.

The harvest in July is the biggest and most profitable.

How long can you store your maize?
Up to a year.

What do you use the maize for?
Only food, some is sold.

What are the biggest problems with your current storage system?
Weevils and not having enough wood. If there is not enough wood in the beginning of drying she has problems with moulds as well as weevils. Transport is also a big problem, they have a lot of different fields were they harvest. Sometimes space problem if big harvest.

What current storage system do you think is best?
She doesn't know of any other storage system.

Would you be open to try a new storage system?
Yes she is willing but concerned about where to get the yeast from.

D6 Pauline

Same answers as previous other (the second wife). Samples were taken from both early and late harvest.

D7 Delphine Teumo, Pauline and Marie, Suzanne Nguofack, Fransisca Nzomo, Madame Noumedem, **Therese Kenfack**

How do you harvest and currently store your maize?
Have just started to harvest twice a year. Maize is put straight on the inner roof (one layer) were it gets sundried; some which lies on top of the kitchen gets smoked. If the harvest is big, the maize is turned once a week. Same procedure during both early and late harvest. After three months (when dry) some of the maize gets peeled and put into pots for quick use. The rest of the maize get the leafs peeled off and is put in a container with insecticide.

Early harvest is the biggest.

How long can you store your maize?
Can keep it until next harvest.

What do you use the maize for?
Food and feed.

What are the biggest problems with your current storage system?
Weevils and moulds when the maize was harvested too wet. No space problem.

What current storage system do you think is best?
Don’t know of any other system.

Would you be open to try a new storage system?
Yes if supplied with starting material for it. She is concerned about how you would peel the maize when wet and whether it would be possible to take out some maize from the sealed container straight away.

D8 Marié Kinkeu

How do you harvest and currently store your maize?
Harvest once, early harvest. Harvest is put straight on the inner roof and gets sundried which takes about 2-3 months. The maize lies in many layers and gets turned once a week. When dry the outer layer is peeled off and the maize is put into bags and insecticide is sprayed on.

How long can you store your maize?
Until next harvest so up to a year.

What do you use the maize for?
Food for the family, some of the spoiled maize goes to feed.

What are the biggest problems with your current storage system?
Moulds, weevils, rodents, storage space and transport which is expensive. If the harvest is big and cannot fit into her house she has to buy bamboo and hang the rest. Space problem! Big losses due to rats and insects!

What current storage system do you think is best?
She doesn't know of any other system.

Would you be open to try a new storage system?
Yes, this new storage system sounds good since she loses approximately half of her harvest to moulds, weevils and rodents.

2. Recipes for medias

DG18
31.5g DG18
220g Glycerol
1000ml distilled H_2O
0.13g Chloramphenicol

TGEA
24g TGEA
1000 ml distilled H_2O

VRBG
38.5g VRBG
1000 ml distilled H_2O

YPD
20g Glucose
20g Peptone
10g Yeast extract
15.6g Agar
1000 ml distilled H_2O
0.13g Chloramphenicol