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Oleana Hedman

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Oleana Hedman

Supervisor: Matilda Olstorpe at the Department of Microbiology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden

Assistant Supervisor: Aziwo T. Niba at the Department of Animal Production, Faculty of Agriculture and Agricultural Sciences, University of Dschang, Cameroon

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Abstract

In sub-Saharan Africa post-harvest losses constitute a major problem. In order to preserve food and feed in a safe and sustainable way new methods need to be investigated and introduced. One system to preserve grain in a secure and energy saving way is by using a biocontrol organism. The yeast strain *Hansenula anomala* J121 is known to inhibit growth of moulds and bacteria from the *Enterobacteriaceae* family in stored cereal grain and is therefore regarded as a biocontrol organism.

In this study *H. anomalas* inhibitory effect on two *Enterobacteriaceae* species (*Salmonella enterica serovar paratyphi B* and *Escherichia coli*) commonly found in stored maize in Cameroon was compared with a local yeast isolate belonging to *Meyerozyma guilliermondii*. Maize was inoculated with *H. anomala/ M. guilliermondii*, *H. anomala/ M. guilliermondii* and *E .coli/S .enterica*, *E. coli/S. enterica* and a control with no inoculation. The maize was then put in test tubes with lids penetrated by a syringe to simulate air leakage and incubated in the dark at room temperature (25°C) for 5 ½ weeks.

No comparison could be made between the yeasts due to increases in log CFU/g maize of *Enterobacteriaceae* in all inoculations.

The study also explored the possibility of introducing a new storage system in Cameroon. Ten farmers in the Nforya-Bamenda (NB) area (North West region) and eight farmers in the Dschang (D) area (West region) were interviewed about their harvest, current problems during storage of maize and their openness to try a new storage system using a biocontrol organism. There were some differences in storage systems between the different areas but the most common way of preserving the maize was drying with smoke. The biggest problems in both areas were insects followed by lack of firewood in NB and transport in D. Farmers in NB were all open to try a new storage system whereas the farmers in D were more skeptical and had a lot of practical questions about the system.

During the interviews samples were taken from the farmers maize storages. The samples from NB were analyzed for the presence of *Enterobacteriaceae*, yeasts, moulds and total number of aerobic bacteria in order to get an overview of the hygienic status. All storages were contaminated with moulds (3.09-6.99 log CFU/g maize) and yeasts (2.96-8.38 log CFU/g maize). Plates with aerobic bacteria were smeared and most plates with substrate selective for *Enterobacteriaceae* had odd dilutions due to contamination. No clear CFU counts could therefore be made. The samples from D were not analyzed due to lack of time.

Contents

Introduction

Providing food for the world's growing population has been and continues to be a big challenge. In the year 2050 an estimated 9.1 billion people will populate the earth. Most of the population growth will be in today's developing countries (FAO, 2009) which is why securing food supplies in these parts of the world is crucial. There are many ways in which to accomplish this, one way is to improve storage of different crops and thereby minimize losses.

The base of the human diet is usually cereal grain. A report from FAO (1999) declared that humans get 50% of their energy and protein directly from it. By eating cattle that was fed cereal grain another 25% of our protein and energy needs can be covered.

One common way to preserve cereal grain is reducing the moisture content (MC) to 13 % to inhibit the growth of pathogenic and spoilage microorganisms such as bacteria and moulds (Jonsson & Pettersson, 2000). Drying cereal grain requires a lot of energy and is therefore not a very cost effective way of conserving the grain.

In order to minimize the energy used when preserving cereal grain other methods have been developed. The grain can be harvested at a MC around 30%, put in an airtight container and fermented. This fermentation depends on a strong population of lactic acid bacteria (LAB) which decrease the pH and produce antimicrobial substances that inhibit growth of moulds and other spoilage microorganisms (Olstorpe & Passoth, 2011). If the MC is too low and there has been air leakage in the container the LAB will not have optimal growth conditions which easily leads to incomplete fermentation and unsafe grain. Since these are two frequent problems during fermentation, ways of improving the system have been investigated.

Studies by Olstorpe et al. (2010a) in Sweden have shown that it is possible to use the yeast *Hansenula anomala* as a biocontrol organism controlling the microbial flora of stored moist grain. The strain *H. anomala* J121 was isolated from stored cereal grain in Sweden. Its characteristics and inhibitory effects on pathogenic and spoilage microorganisms have been studied for many years (Schnürer & Jonsson, 2011).

In sub-Saharan Africa, where storage with a biocontrol organism not yet has been introduced, 70% of a normal income comes from crop production (The World Bank, 2011) which means that crop losses have a huge impact on the household economy. Maize is an important staple crop in sub-Saharan Africa (Nuss &Tanumihardjo, 2010). In Cameroon, where the present study took place, the annual production of maize is 1.2 million tons which makes it the third biggest crop grown in the country and the mostly cultivated cereal grain (FAO, 2008). Two-thirds of Cameroons inhabitants consume maize regularly because of it being a relatively cheap crop.

Maize is not a very nutritious staple food, some of the essential amino acids such as tryptophan and lysine are absent and the minerals iron and iodine are deficient (Nuss &Tanumihardjo, 2010).

Studies have shown that the protein content increased as well as the value of the protein when inoculating grain with *P. anomala* in Sweden (Olstorpe et al., 2010a). *H. anomala* has also been recorded to have a high phytase activity (Olstorpe et al., 2009). Phytic acid in maize binds up (chelates) calcium, zinc, and iron, three important minerals. With the help of phytase from *H. anomala* more of these minerals could become available. Even though the increase in protein value was not high enough for Swedish standards, together with the high phytase activity it could be a significant contribution to a nutritionally poor diet in a developing country such as Cameroon (Nuss & Tanumihardjo, 2010).

Bacteria belonging to the *Enterobacteriaceae* family have been detected in stored cereal grain (Lyberg et al., 2007). This family of bacteria contains several pathogens for example species of *Salmonella*, *Shigella* and *Escherichia*. Especially in developing countries, where health of humans and animals is not optimal in most cases, *Enterobaceriaceae* could be a problem. In fact as much as 70% of diarrheal cases in developing countries are associated with pathogens from food (Kimmons et al., 1999).Two studies, both made in Sweden, Olstorpe et al., (2010a) and Furman (2011) have shown that *H. anomala* can inhibit growth of bacteria from the *Enterobacteriaceae* family.
The present study was partly about investigating *H. anomalas* inhibitory effects on two species of *Enterobacteriaceae* commonly found in maize storages in Cameroon compared to a local yeast isolate (*Meyerozyma guilliermondii*). This yeast is mostly known as a biocontrol for fruits. Marginal inhibitory effects on mould growth on wheat have been observed in a model grain storage system (Schnürer &Jonsson, 2011) but possible inhibition of *Enterobacteriaceae* has never been studied.
Another part was taking samples from different farms storages and make CFU counts of *Enterobacteriaceae*, yeasts, moulds and aerobic bacteria, to test for possible contaminations of the storages. During sampling of maize storages farmers where interviewed about their storage problems and willingness of trying a new storage system in order to explore their interest in using a biocontrol organism.

Materials and Methods

The methods consist of three different parts. The first part is a biocontrol test comparing *M. guilliermondii* with *H. anomala* in inhibitory effect on bacteria from the *Enterobaceriaceae* family. The second and third part consists of a hygienic evaluation of maize storages in the North West and West region of Cameroon and interviews with maize farmers.

All laboratory work was done at the Animal Health Laboratory, Department of Animal Production, Faculty of Agriculture and Agricultural sciences, University of Dschang, Dschang, Cameroon.

Preparation of microorganisms

M. guilliermondii and *H. anomala* were sent from the Department of Microbiology at the Swedish University of Agriculture (SLU), Uppsala, Sweden as cultures on Yeast extract Peptone Dextrose (YPD) medium. The composition of all media used in this lab can be found in appendix 1. Before inoculation the isolates were pure cultivated overnight in room temperature (25°C). *Salmonella enterica serovar paratyphi B* and *Escherichia coli* were originally obtained from the Pasteur Center (Institute Pasteur) in Yaoundé and were provided by Madamé Briget Katté at the University of Dschang, Cameroon. Both *Enterobacteriaceae* species had been stocked in tryptose broth and were pure cultivated overnight in 30°C before inoculation.

Biocontrol

Hygiene analysis of maize used for biocontrol test

Maize (3kg) was soaked for a week in boiled tap water (900 ml) to increase the MC to approximately 30 % which is average at early harvest.
The exact MC was calculated by weighing a soaked triplicate of maize and let it stand in an oven at 75 °C for three days before weighing it again and calculating the difference, it was assumed that the weight then was constant. A triplicate of 20 g maize and 180 g peptone water was vigorously shaken for some time in stomacher bags before 10 ml was taken out and dilution series with peptone water were made. Dilutions were spread onto Dichloran Glycerol 18 % agar (DG18), YPD, Violet Red Bile Glucose agar (VRBG) and Tryptone Glucose Extract Agar (TGEA) according to recipes in appendix 1. Colony forming units (CFU)/g were calculated for moulds, yeasts, *Enterobacteriaceae* and aerobic bacteria on the different media after appropriate incubation times and temperature.

Inoculation

Maize was inoculated with approximately 10^5 CFU/g of only *H. anomala* and only *M. guilliermondii*, it was also inoculated with 10^3 CFU/g of only *S. enterica* and *E. coli*. The cell numbers were determined in two ways, first by the use of a Bürcker cell counting chamber and later (after inoculations) by CFU counts on specific substrates. Incubation times and media used for the specific microorganisms are shown in table 1. The determination of cell numbers by the Bürcker cell counting chamber was in some cases done one day before inoculations.

Table 1. *Incubation times, temperatures and specific substrates used before counting CFU*

Microorganism	Media	Incubation time (days)	Incubation temperature (°C)
E. coli	VRBG	1.5	30
S. enterica	VRBG	1.5	30
P. anomala	YPD	2	RT*(25)
M. guilliermondii	YPD	2	RT(25)

**RT = room temperature*

Inoculated maize and a control sample were put into test tubes, approximately 10 g in each and a lid was put on with a syringe penetrating to simulate air leakage (Pettersson & Schnürer, 1995). In addition to the above mentioned inoculations combined inoculations were made as shown in table 2. These inoculations were also put into the same type of test tubes. All tests were made as triplicates. The test tubes were incubated in the dark at 25°C for 5 ½ weeks. The concentrations of the different microorganisms were then determined in the same way as at the start of inoculation.

Table 2. *Combined inoculations (the concentration is an estimate made from cell counts in a Bürcker cell counting chamber)*

Concentration (log CFU/g)		
5.0	***H. anomala*** **co-inoculated with either:**	***M. guilliermondii*** **co-inoculated with either:**
3.0	*E. coli*	*E. coli*
3.0	*S. enterica*	*S. enterica*

Evaluation of maize storages

Ten farmers in the Nforya-Bamenda (NB) area (North West region of Cameroon) and eight farmers in the Dschang (D) area (West region of Cameroon) were interviewed about their current storage system of maize. After briefly explaining about using a biocontrol during storage the farmers were asked these questions: *How do you harvest and currently store your maize? How long can you store your maize? What do you use the maize for? What are the biggest problems with your current storage system? What current storage system do you think is best? Would you be open to try a new storage system?*
An interpreter was used when interviewing farmers in the D area. The full interviews can be found in appendix 2. Samples, in triplicates were taken from each farmer's maize storage.
The samples from the NB area were analyzed for the presence of moulds, yeasts, *Enterobacteriaceae* and aerobic bacteria, using the same method described under "Hygiene analysis of maize used for biocontrol test" except 5 g maize was mixed with 45 g peptone water.

Results

Biocontrol

Hygiene analysis of maize used for biocontrol test

The MC of the soaked maize used in the biocontrol test was 19 %. The MC before being soaked was 5.6 %.

No yeasts or *Enterobacteriaceae* were detected in the hygiene analysis of the maize used for the biocontrol test at the start. A lot of the plates with

YPD medium (specific for yeast) were contaminated with moulds. After 5 ½ weeks 6.55 log CFU/g maize (n = 3) with the standard error 0.30 of *Enterobacteriaceae* could be seen but no yeast.
The mean log CFU/g maize value at the start for aerobic bacteria was 3.40 and 4.04 for moulds with the standard errors of mean 0.10 and 0.67 respectively. The mean CFU/g maize of moulds had increased to 8.44 with the standard error of mean 0.08 at the end of inoculation. The TGEA plates with aerobic bacteria were too contaminated to obtain any results.

Inoculations

CFU/g maize at start of inoculation and after 5 ½ weeks can be seen in table 3, the treatment called control is the yeast or *Enterobacteriaceae* species without co-inoculation. The concentration of *Enterobacteriaceae* was higher than the estimation in the Bürcker cell counting chamber at the start of inoculation and the concentration of *H. anomala* was lower. Both yeasts and *Enterobacteriaceae* had increased in concentration in all treatments after 5 ½ weeks. None of the treatments showed any significant decrease in *Enterobacteriaceae* compared to the control.

Table 3. *The mean log CFU/g (n = 3) value of the two yeast and* Enterobacteriaceae *species at start of inoculation and after 5 ½ weeks in the different treatments. The treatment called control is the yeast or* Enterobacteriaceae *species without co-inoculation*

		Log CFU/g maize		**Standard errors of mean**	
	Treatment*	**Start**	**5 ½ weeks**	**Start**	**5 ½ weeks**
H. anomala	C	4.89	7.79	0.10	1.51
	P+E	4.89	7.77	0.10	0.72
	P+S	4.89	6.73	0.10	0.35
M.guilliermondii	C	5.18	6.76	0.19	0.20
	M+E	5.18	8.67	0.19	1.00
	M+S	5.18	7.04	0.19	0.27
S. enterica	C	3.81	7.33	0.11	0.37
	P+S	3.81	6.57	0.11	0.05
	M+S	3.81	6.96	0.11	0.41
E. coli	C	3.74	6.92	0.29	0.29
	P+E	3.74	6.68	0.29	0.07
	M+E	3.74	6.59	0.29	0.47

**C=control, P+E=* H.anomala+E.coli, *P+S*=H.anomala+S.enterica, *M+E*=M.guilliermondii+E.coli, *M+S*=M.guilliermondii+S.enterica

Evaluation of maize storages

There was a big difference between the farmers in the Nforya-Bamenda (NB) area and the ones in Dschang (D). Two of the farmers in D were interviewed at the same time and had exactly the same answers (they were living on the same farm) therefore only seven of the eight interviews will be taken into account. In total 17 farmers were interviewed. The questions asked during the interviews will be gone through one by one.

How do you harvest and currently store your maize?

All farmers harvested during the rainy season (early harvest), six also harvested a second time during the dry season. Since the maize harvested during the rainy season has an average MC of 30 % and thus is most suitable for a biocontrol, only answers concerning the early harvest will be presented as results.

There were three common ways to store the maize. One was by hanging/laying it from/on the inner roof and then drying it by smoking while unpeeled. If the maize was laid on the inner roof it had to be turned once or twice a week. Drying by smoking took 2-3 months. Another method was laying the maize on the inner roof and letting the heat from the sun on the outer roof dry it, which also required turning the maize once or twice a week and took 2-3 months. The third way of storing was by putting it in an airy shed, called a crib, and letting the wind dry it. When the maize was dry it was generally peeled, sprayed with insecticide and put into sacks or plastic containers for longer storage. When using cribs, the maize could stay in them during the whole storage period but they were still sprayed with insecticide. The different storage systems can be seen in figure 1-2.

Figure 1. *Storage by hanging from the inner roof and smoking (to the left). Storage in cribs (to the right).*

Figure 2. *Storage by "sun drying" leftover peelings can be seen on the inner roof.*

As shown in figure 3 the most common way of storing in both NB and D was by smoking (11 farmers). The system with cribs was only used in NB and only farmers in D sundried and sundried/smoked their maize.

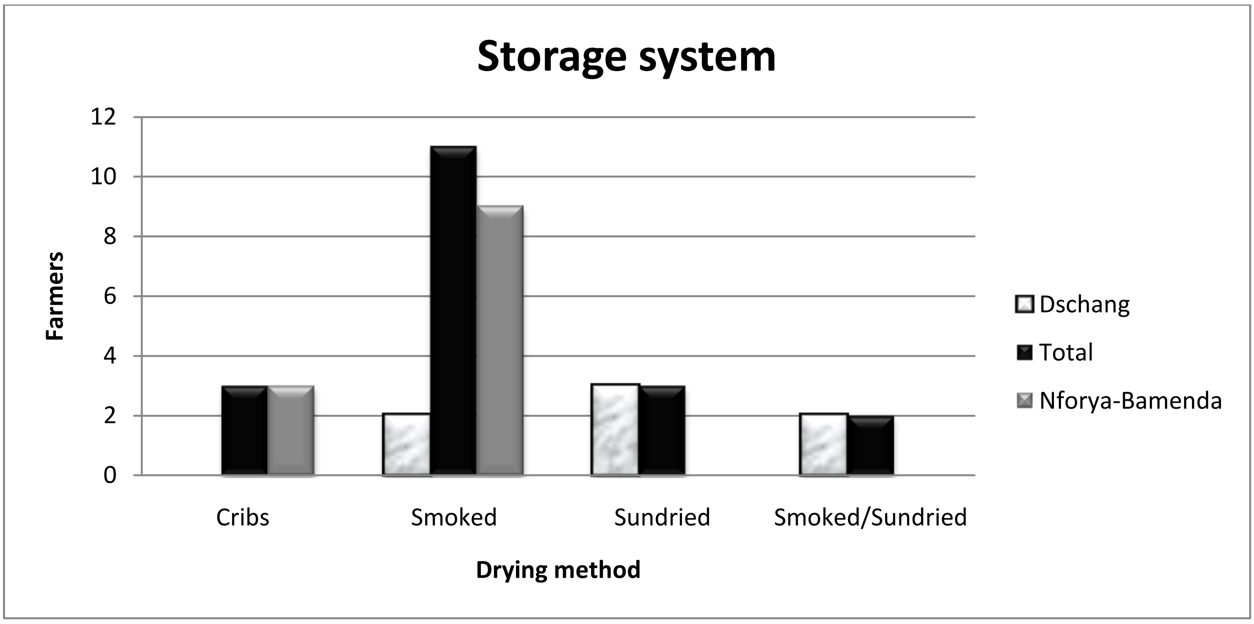

Figure 3.*Storage systems used in the two different areas and in total.*

What current storage system do you think is best?

The most preferred storage system in NB was cribs (nine farmers), in D most farmers (four out of seven) only knew of their own system so they did not know what system they would prefer.

How long can you store your maize?

Only one of the farmers in NB could store their maize for more than six months whereas all farmers in D could store their maize up until the next harvest so approximately a year. Of the NB farmers four could store it up to six months and four could store it for less than three months.

All farmers in NB used the maize both as food and feed for poultry or cattle. In D, two farmers only used it as food and the rest used some of the spoiled maize as feed.

What are the biggest problems with your current storage system?

There were many problems mentioned, all of them can be seen in figure 4. According to 15 farmers the most common problem was insects infecting the maize. The need for firewood was also a big concern to eight farmers. In D transporting the harvest to the house was a big issue to four of the seven farmers as well as rodents eating the maize and mould spoiling it. Two farmers in NB were concerned about all labour connected to tying/turning the maize during smoking. Only three of the NB farmers saw moulds as a big problem. Having enough storage room was a problem in both NB and D.

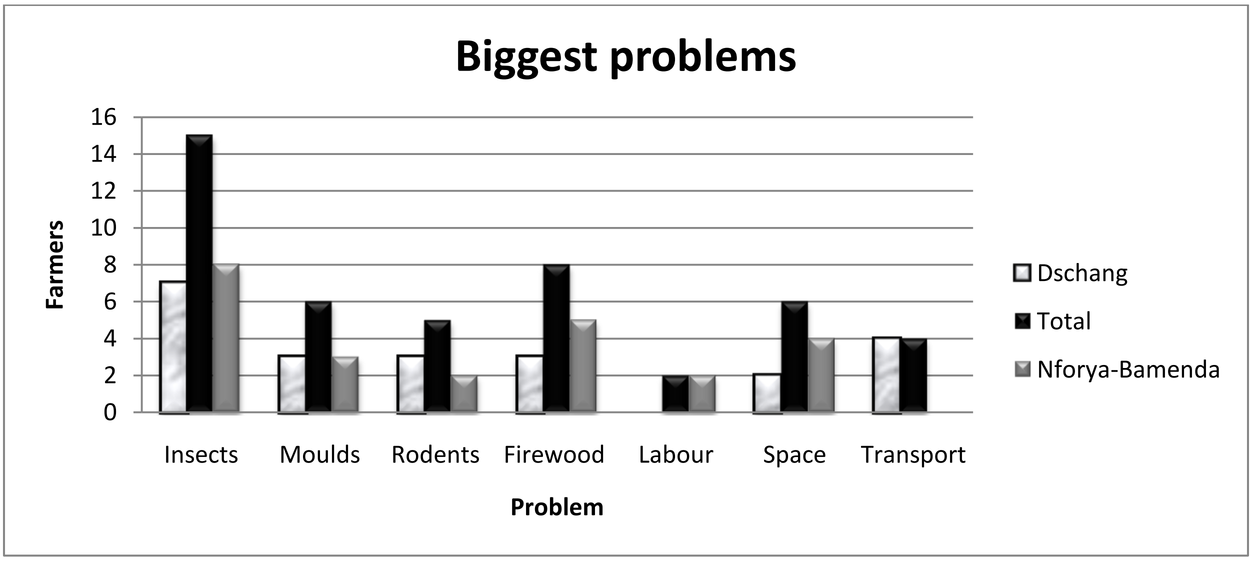

Figure 4. *The biggest problems concerning maize storage in the two different areas and in total.*

Would you be open to try a new storage system?

All farmers in NB said they would be open to try a new storage system. In D the farmers were more skeptical and wanted to see if it worked before trying it themselves. Questions about difficulties with threshing the wet maize and where they would get suitable containers were raised. One farmer said no because she always sold some of her maize as seeds for next year and by using the biocontrol system she would probably not be able to do that.

Hygiene status of maize storages

The samples from the farms were taken in April/May when the farmers who only harvested once already had finished their maize. The sample material was therefore ranging from burned maize leafs to dried stored maize seeds and combinations of these which gave very varying results.

The CFU on the TGEA plates used to evaluate the occurrence of aerobic bacteria were all too numerous to count (TNTC) and mostly smeared which made it impossible to get a reliable result. On the YPD media the yeasts were mostly TNTC, the CFU was therefore counted on DG18 where clear colonies could be seen. No clear CFU count for the occurrence of *Enterobacteriaceae* could be done because of irregular plated dilutions caused by contamination of other bacteria but in seven of the samples *Enterobacteriaceae* were detected. The mean log CFU/g maize (n=3) value for yeasts and moulds can be seen in table 5. Farms with a higher concentration of yeasts seemed to have a higher concentration of moulds as well.

Table 5. *Concentrations of yeasts and moulds in the samples from the farms in NB. The samples from D could not be analyzed due to lack of time*

	Log CFU/g maize		Log CFU/g maize	
Farm	Yeast	Standard errors	Moulds	Standard errors
NB1	6.15	1.86	4.45	0.54
NB2	4.90	1.96	3.24	0.24
NB3	2.96	0.20	3.56	0.34
NB4	3.75	-*	3.18	0.32
NB5	8.38	1.43	6.95	0.78
NB6	8.15	3.11	6.03	1.86
NB7	5.04	0.58	3.09	0.08
NB8	4.43	0.87	3.29	0.75
NB9	8.07	0.61	6.99	1.02
NB10	4.27	0.49	3.86	0.66

**No mean was calculated because CFU/g was only based on one dilution series.*

Due to lack of time the samples from Dschang could not be analyzed.

Discussion

Biocontrol

Part of the purpose with this study was to evaluate *M. guilliermondii* as a potential inhibitor of *Enterobacteriaceae* during moist grain storage. Conclusions regarding this could not be made due to the fact that *H. anomala*, which was supposed to be the comparable microorganism, gave no reliable result. After 5 ½ weeks both *E. coli* and *S. enterica* had increased in CFU just as much in the control sample as in both treatments with the two different yeasts.

We only managed to increase the MC to 19 % in the maize used for inoculation with the different microorganisms even though it was soaked for a week. The maize was really dry to start (MC=5.6%) which probably is why it was impossible to increase to an appropriate level. A MC of 19% is

not very favorable for fermentation (by LAB) but for mould growth according to Olstorpe et al. (2010b). All minisilos were visibly infected with a lot of different mould species after 5 ½ weeks. The hygiene analysis of the maize used for the biocontrol test showed a high log CFU/g value for moulds (8.44) which confirms that the MC was profitable for them. The low MC also makes it difficult for LAB to proliferate.
The inability to increase the MC to 30% together with all the mould growth are two possible reasons why the study could not demonstrate any inhibition of *Enterobacteriaceae* by *H. anomala* even though two previous studies on cereal grain have shown this (Olstorpe et al., 2010a; Furman, 2011). Also the inoculated concentration of *Enterobacteriaceae* was too high and the concentration of *H. anomala* too low at the start which could have made it difficult for the yeast to colonize properly. A possible reason for the higher concentration determined by CFU counts could be that some of the inoculations took place the day after cell counts were made in the Bürcker chamber and the microorganisms then could have increased in numbers. Another dissimilarity is that this project was done on maize and not any of the crops commonly grown in Sweden (oats, barley and rye) which could make a difference. Furthermore inhibition below the detection level (CFU 10^1) of *Enterobacteriaceae* has only been shown after 2 months inoculation (Furman, 2011) which could mean that the 5 ½ weeks long inoculation was too short to make a difference. But Furman (2011) study also showed a decrease in CFU of *Enterobacteriaceae* already after two weeks which contradicts that argument. The test tubes used during the inoculation were very small which made it difficult to mix the maize properly. This could also have been a contributing factor for not getting a reliable result.
The presence of *Enterobacteriaceae* in the hygiene analysis of the maize used for the biocontrol test after 5 ½ weeks is hard to explain, maybe the dilutions plated at the start were too high to show any colonies. It is also possible that the biocontrol hygienic analysis maize was contaminated before being put in the minisilos.
In order to assess if *M. guilliermondii* could be a biocontrol organism another study would have to be made. Preferably maize should be taken fresh from harvest during the rainy season to get a more accurate MC and less mould contamination. Also more studies on *H. anomala* as a biocontrol organism in maize would be interesting. This study had no means to measure changes in mineral availability or in protein values of the maize but these are also two things that could be considered when making a new study.

In the hygiene analysis of the NB farmers storage no CFU values for *Enterobacteriaceae* on VRBG could be calculated due to contaminations by other bacteria/yeasts. However most of the samples did contain *Enterobacteriaceae* which confirms that a problem exists and more studies on inhibition of this family of bacteria are required.

Evaluation of maize storages

Answers from farmers in the two different areas varied a lot in some

questions. This shows that some storage systems and ways of seeing things are very local. The farmers in D lived closer to/in the city which made transport from the field difficult while the NB farmers lived more on the countryside and therefore had other issues.
All farmers harvested during the rainy season and the farmers who harvested twice a year always said the early harvest was the biggest. This is probably why all farmers chose to harvest during the rainy season even though a lot of labour and firewood went into drying it.
Surprisingly few saw moulds as a big problem (six farmers in total), one reason could be that insects are much more visible. Earlier studies have shown that problems with insects (weevils) could be an indication of mould infection since the insects makes the maize more vulnerable (Sone, 2001).
In D more farmers saw moulds as an issue which could be due to their system of sun drying which probably was a more favourable environment for mould growth.
When using smoke for drying a lot of firewood, which is hard to get by and expensive, was used and it seemed to be very labour intensive having to tie up all the maize or turn it if it was laying on the inner roof. These two factors were probably the biggest contribution to the fact that most farmers in NB preferred the cribs system.
The farmers using the cribs had other problems though such as rodents, moulds and termites which could be why all farmers in NB, no matter which system they used, where open to try a new storage system.
Using the new system would be a lot less labour-intensive since the early harvest could just be peeled, threshed and put straight into plastic containers. The biocontrol yeast could then be sprayed on it prior to sealing which would keep the insects out.
The farmers in D were a lot more skeptical to try a new system and seemed to want to know if it really worked before trying it themselves. Reasons for being more reserved could be not having a big harvest in the first place and then being afraid of losing some of it if the new storage system would fail. Another bias when talking to the farmers in D was having to use an interpreter which opened up for a lot of misunderstandings. The farmers in D also in general used their maize more as food than as feed which could contribute to their doubts since the biocontrol storage would make the maize into a new product that would be easier to introduce as feed. Grain inoculated with *H. anomala* has previously only been used as feed for cattle (Olstorpe et al., 2010a). Some practical questions like; how they would thresh the seeds when the maize was wet, where they would get suitable storage containers and who would provide the yeast were raised by the D farmers. No good threshing method for wet maize is known to the author of this report but it could be important to search for one and think about the practical distributional issues before trying to introduce this new system.

Storing the maize by fermenting and using a biocontrol would save a lot of space as well which six farmers from both areas also thought was a big problem. It was only halfway through the interviews in NB we started asking if the farmers thought it was difficult to fit all the harvest which

could indicate that even more farmers, if asked, would see that as a big problem.
Trouble with not having enough storage room is important to consider since a lot of today's projects aims to increase the yield of maize (CGIAR, 2011). If the farmers still cannot store it in a proper way a maize variety with a higher yield will make no difference.
There are also many health aspects to consider when it comes to smoking the maize. The people, mostly women and children, who have to stay in a very smoky environment for many hours a day, are exposed to a lot of air pollution. The WHO (2006) showed that air pollution greatly increases the risk of pneumonia in children less than five years old and of women getting chronic obstructive pulmonary disease. In fact, indoor smoke contributed to almost 400 000 deaths in Sub-Saharan Africa in 2002. Since the farmers normally smoke the maize in the kitchen which they anyway would use when cooking, this new storage system may not be the full solution to the problem. It might decrease the intensity of exposure to smoke though which would help a little.

Hygiene status of maize storages

In all storages there was a lot of moulds and yeast. Where there were high numbers of yeasts there were also high numbers of moulds. This just indicates that some farms had higher contaminations than others. Since the yeasts had to be counted on DG18, which is not their optimal growth medium, the actual concentrations could have been higher.
To get a better overview it would have been good to have gotten some reliable results on the total number of aerobic bacteria and *Enterobacteriaceae*. It would also have been interesting to identify some of the *Enterobacteriaceae* species found to see which are most common. The sample material varied a lot between the different farms which also makes it hard to draw any real conclusions. The only thing that can be said is that all farms had a lot of moulds and yeasts no matter what the samples looked like and no matter whether the farmers thought it was a problem. Even on smoked, black leftover peelings mould contamination were observed.
In a study by Ny et al. (2011), hygiene analyses were made at different time points on maize from two farms, one in D and one in NB during the last year. Some of the microbial flora and changes in it was identified. The farms from the two different areas had very different microbial floras, both storages contained *Enterobacteriaceae* but one significantly more than the other. This showed that *Enterobacteriaceae* presents a problem which was confirmed in the present study due the occurrence of this family of bacteria on most farms.

Conclusions

No conclusions could be drawn regarding *M. guilliermondiis* ability to inhibit the growth of *Enterobacteriaceae*. A study using fresh maize harvested during the rainy season could maybe get a better result.

Most farmers, both in Nforya-Bamenda (NB) and Dschang (D) were open to try a new storage system even though the farmers in NB seemed to be less skeptical. When trying to introduce the new biocontrol system it would therefore probably be easier to start in the NB area but before launching it, practical issues are important to consider. Many of the problems such as insects, firewood, rodents and moulds farmers had might be solved by the new storage system.

Most of the samples from the farmers in the NB area clearly contained *Enterobacteriaceae* even though no CFU count could be made. This confirmed the fact that more studies on possible inhibitory microorganisms needs to be made.

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References

CGIAR (2011) Consultative Group on International Agricultural Research. 40 Findings on the Impacts of CGIAR Research 1971-2011

FAO (2008) Food and Agriculture Organization of the United Nations http://faostat.fao.org/DesktopDefault.aspx?pageid=339&country=32&lang=en 2011-03-31

FAO (2009) How to feed the world 2050. Food Nutrition and Agriculture

FAO (1999) Fermented Cereals. A Global Perspective. FAO Agricultural service bulletin no. 138

Furman G. (2011). Inhibition of *Enterobacteriaceae* by *Pichia anomala* during moist grain storage. Uppsala: Swedish University of Agricultural Sciences. SLU-MIKRO-EX-11/2-SE

Jonsson N. & Pettersson H. (2000). Utvärdering av olika konserveringsmetoder för spannmål-baserad på analyser av hygienisk kvalitet (in Swedish). Report no 263. Swedish Institute of Agricultural and Environmental Engineering (JTI)

Kimmons J.E.,Brown K.H., Lartey A., Collison E., Mensah P.P.A. & Dewey K. G. (1999). The effects of fermentation and/or vacuum flask storage on the presence of coliforms in complementary foods prepared for Ghanaian children. International Journal of Food Sciences and Nutrition (1999) 50: 195-201

Lyberg K., Olstorpe M., Passoth V., Schnürer J. & Lindberg J.E. (2007). Biochemical and microbiological properties of a cereal mix fermented with whey, wet wheat distillers' grain or water at different temperatures. Animal Feed Science and Technology 144: 137-148

Nuss T. E. & Tanumihardjo A. S. (2010) Maize: A Paramount Staple Crop in the Context of Global Nutrition. Comprehensive Reviews in Food Science and Food Safety. 9: 417-436

Ny S., Niba A. T., Olstorpe M. (2011)Hygiene evaluation of maize from harvest to storage. Oral presentation 2011-05-26 at the "Third Life Science Conference" University of Dschang, Cameroon

Olstorpe M., Borling J., Schnürer J. & Passoth V. (2010a). The biocontrol yeast *Pichia anomala* improves feed hygiene during storage of moist crimped cereal grains under Swedish farm conditions. Animal Feed Science and Technology 156: 47-56

Olstorpe M., Schnürer J. & Passoth V. (2010b). Microbial changes during storage of moist crimped cereal barley grain under Swedish farm conditions. Animal Feed Science and Technology 156:37-46

Olstorpe M. & Passoth V. (2011) *Pichia anomala* in grain biopreservation. Antonie van Leeuwenhoek 99:57–62

Olstorpe M., Schnürer J. & Passoth V. (2009). Screening of yeast strains for phytase activity. FEMS Yeast Research 6:3-13

Petersson S. & Schnürer J. (1995) Biocontrol of mould growth in high-moisture wheat stored under airtight conditions by *Pichia anomala*, *Pichia guilliermondii*, and *Saccharomyces cerevisiae*. Applied Environmental Microbiology 61:1027–1032

Schnürer J. & Jonsson A. (2011). *Pichia anomala* J121: a 30-year overnight near success biopreservation story. Antonie van Leeuwenhoek 99:5–12

Sone J. (2001). Mold growth in maize storage as affected by compound factors: Different levels of maize weevils, broken corn and foreign materials, and moisture contents. Journal of Asia-Pacific Entomology 4: 17-21

The World Bank (2011). Missing Food: The case of postharvest grain losses in sub-Saharan Africa. Report no. 60371-AFR

WHO (2006) Fuel for life: Household energy and health

Appendix 1

Recipes of the media used

YPD

Glucose 20 g
Peptone 20 g
Agar agar 15.6 g
Yeast extract 10 g
Cloramphenicol 0.1 g
Distilled water 1L

The medium was mixed and sterilized by autoclaving in 121 °C for 15 min. Approximately 20 g medium were plated on each petri dish. After solidification 100 µl of sample solution was spread on.

DG18

DG18 31.5 g
Glycerol 220 g
Cloramphenicol 0.13 g
Distilled water 1 L

The medium was mixed and sterilized by autoclaving in 121 °C for 15 min. Approximately 20 g medium were plated on each petri dish. After solidification 100 µl of sample solution was spread on.

VRBG

VRBG 38.5 g
Distilled water 1 L

The medium was freshly made up every sampling day. It was boiled for 2 minutes until all agar was dissolved then cooled to 47 °C and used within 3 hours. Approximately 20 g medium was poured into each petri dish and 1 ml sample solution. After solidification an overlay of approximately 10 g medium was poured.

TGEA

TGEA 24 g
Distilled water 1 L

The medium was freshly made up every sampling day.The medium was mixed and sterilized by autoclaving in 121 °C for 15 min. It was then stored in incubator, 47 °C, prior to pouring the plates. Approximately 20 g medium and 1 ml sample solution per plate. After solidification an overlay of approximately 10 g of medium was poured.

Appendix 2

Mini interviews with farmers:

Farmers in Nforya-Bamenda

NB1 Shu Martin Amaboh

How do you harvest and currently store your maize?
Harvest twice. 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 a crib 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.

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

NB2 Manka'a Veronica

How do you harvest and currently store your maize?
Harvest once. Early harvest: the maize goes straight into the barn and gets 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.

What current storage system do you think is best?
Cribs.

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

NB3 Andrew Shu Niba

How do you harvest and currently store your maize?
Harvest twice. Early harvest: Maize gets taken from the field and 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 hung 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. The late harvest is easier to sell 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.

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.

NB4 Dorothy Awah Tacheh

How do you harvest and currently store your maize?

Harvest once, early harvest. 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 consumption the maize gets washed, dried and grinded. If the harvest is big the maize also goes up in the barn (inner roof) 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 insecticide.

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.

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

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

NB5 Nkwenti Peter Ajini

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 insecticide 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.

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 insecticide lasts for three months so as long as you apply new insecticide 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.

What current storage system do you think is best?

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

NB6 Lilian Nasi

How do you harvest and currently store your maize?
Harvest once. The maize gets put in the barn (inner roof) 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 insecticides 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.

NB7 Che Chrisantus Che

How do you harvest and currently store your maize?
Harvest twice. Early harvest: Maize gets put in the barn (inner roof) 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.

NB8 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.

NB9 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 (inner roof) 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.

NB10 Nimang Pius 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 (inner roof) 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 and stem borers.

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.

Farmers in 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 year's harvest.

What are the biggest problems with your current storage system?
Transporting the maize, firewood, weevils if the maize has dried and not been put in plastic containers straight away.

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

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.

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/sundried. 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.

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

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.

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.

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.