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Occurrence and microbial diversity of yeasts during spontaneous fermentation of mawè, a cereal-based dough produced in West Africa

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Introduction

Mawè is an uncooked cereal-based fermented dough largely consumed in Benin, West Africa. Mawè is a result of spontaneous fermentation of yeasts and lactic acid bacteria for 24-48h. The occurrence and the diversity of yeasts involved in mawè fermentation were investigated in this study.

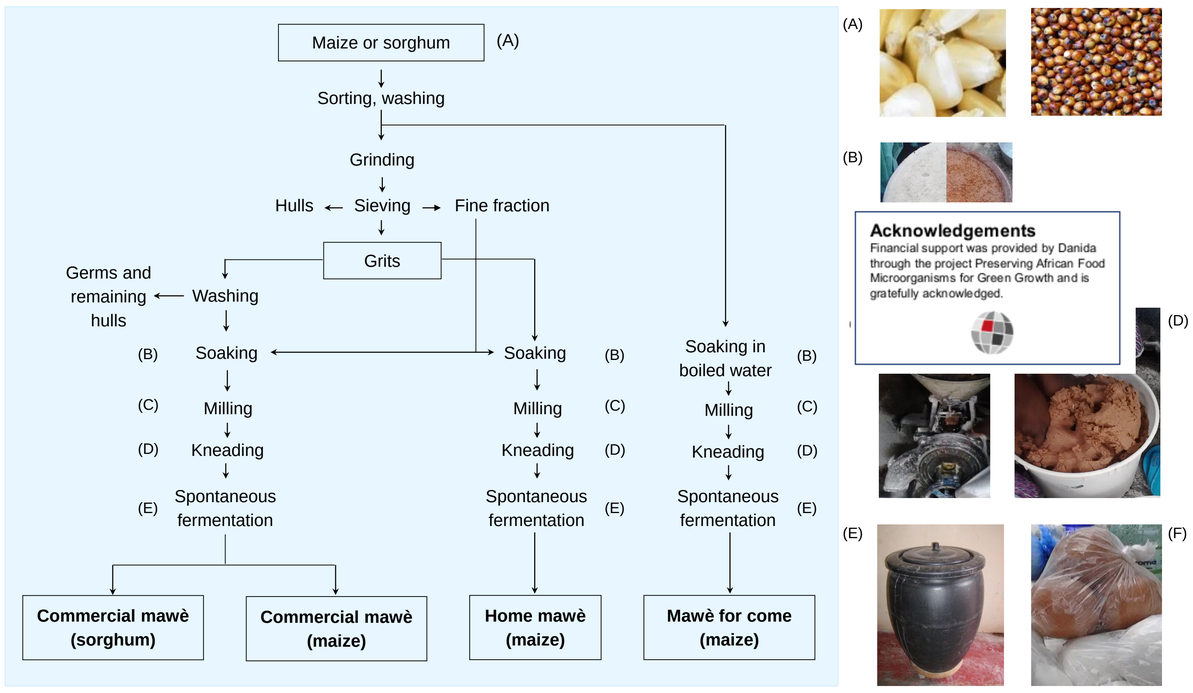

Fig. 1. Flow diagram for production of the four kinds of mawè: commercial mawè (sorghum), commercial mawè (maize), home mawè (maize) and mawè for come (maize). Duration of the spontaneous fermentation is 24-48h. Pictures to the right represent the different steps indicated with capital letters on the flow diagram (A-E). (F) depicts moulded mawè ready for sale.

Materials and methods

Four kinds of mawè produced in the Southern Benin were studied, i.e. commercial mawè (sorghum), commercial mawè (maize), home mawè (maize) and mawè for come (maize) (Fig. 1). Two productions sites for each type of mawè were sampled five times during the fermentation at 0h, 6h, 12h, 24h and 36h.

Isolated yeasts (334) were grouped by $(GTG)_5$-based repetitive PCR (rep-PCR) followed by D1/D2 domain 26S rRNA gene sequencing. *Kluyveromyces marxianus* were unambiguously identified to species level by restriction fragment length polymorphism of internal transcribed spacer regions (ITS) in addition to sequencing.

Cluster analysis of $(GTG)_5$-based rep-PCR fingerprints were calculated using Dice's coefficient of similarity with unweighted pair group method using arithmetic average clustering algorithm (UPGMA).

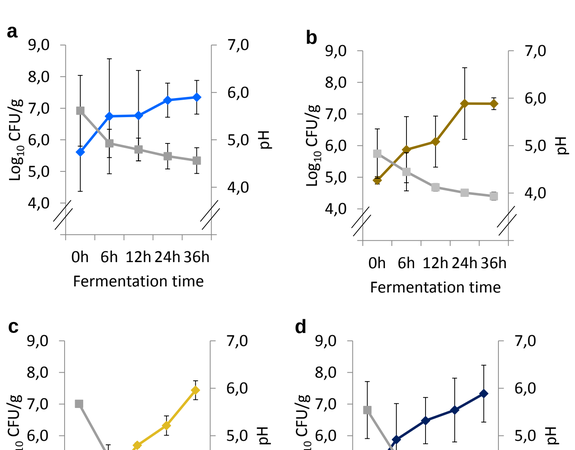

Fig. 2. Microbial counts and pH change (■) during mawè fermentation in the four different kinds of mawè: (a) commercial mawè (sorghum), (b) commercial mawè (maize), (c) home mawè (maize) and (d) mawè for come (maize).

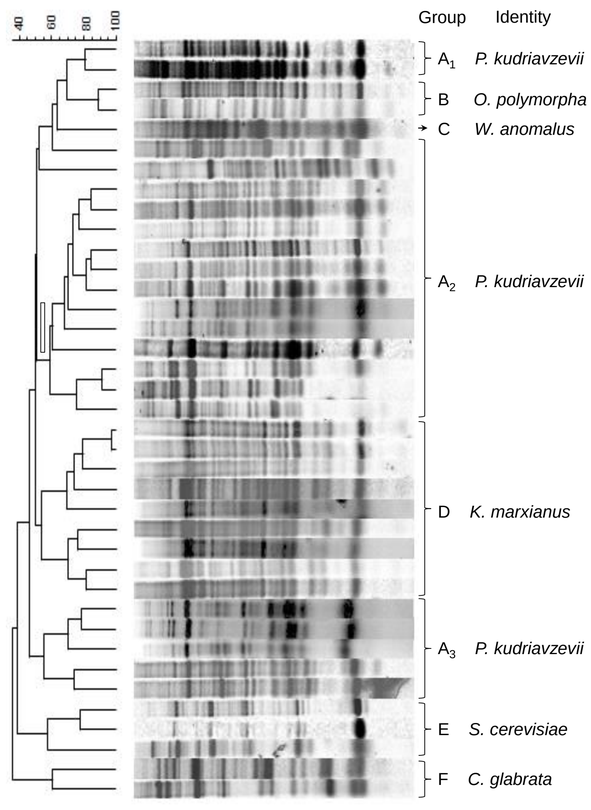

Fig. 3. Dendrogram obtained by cluster analysis of $(GTG)_5$-based rep-PCR fingerprints of yeasts isolated during spontaneous fermentation of the four kinds of mawè. The dendrogram is based on Dice's coefficient of similarity with the unweighted pair group method using arithmetic average clustering algorithm (UPGMA). Isolates were subsequently identified by sequencing of 26S rRNA gene. Only a representative subsample of the isolates is shown.

Table 1. Yeast groups, identify, numbers of isolates and percentages.

Group	Identity	Number of isolates	Percentage
A_1	*P. kudriavzevii*	4	1%
A_2	*P. kudriavzevii*	200	60%
A_3	*P. kudriavzevii*	18	5%
B	*O. polymorpha*	7	2%
C	*W. anomalus*	1	0.3%
D	*K. marxianus*	83	25%
E	*S. cerevisiae*	17	5%
F	*C. glabrata*	4	1%

Results

Generally, yeast counts increased in the four kinds of mawè during the spontaneous fermentation from 4.8±0.8 $\log_{10}$ cfu/g at 0h of fermentation to 7.4±0.4 $\log_{10}$ cfu/g at the end of fermentation (36h) (Fig. 2). The highest increase was observed for home mawè (maize) where the yeast counts were 4.2±0.6 $\log_{10}$ cfu/g at 0h of fermentation and increased to 7.4±0.3 $\log_{10}$ cfu/g at the end of fermentation (Fig 2c). The pH of the mawè products generally decreased from 5.4±0.5 at 0h to 4.1±0.3 after 36h of fermentation. The mawè product with the lowest pH was mawè for come (maize) where pH dropped from 5.5±0.6 at 0h of fermentation to 3.9±0.1 at the end of fermentation (Fig 2d).

The yeast isolates grouped into eight groups based on the cluster analysis of the $(GTG)_5$-based rep-PCR and were identified to six yeast species (Fig. 3). The most abundant and diverse yeast species isolated from the mawè products were *Pichia kudriavzevii*, which were presented by three groups i.e. A_1, A_2 and A_3 (Fig. 3), indicating that a variety of *P. kudriavzevii* biotypes were involved in the spontaneous mawè fermentations. Of the isolated yeasts *P. kudriavzevii* comprised 66%, *Kluyveromyces marxianus* 25% and *Saccharomyces cerevisiae* 5%, respectively. A minor part of the yeasts were identified as *Ogataea polymorpha* (metylotrophic yeast originally isolated from soil, probably introduced from raw materials), *Candida glabrata* and *Wickerhamomyces anomalus* (together comprising <4%) (Table 1).

Succession of the yeasts in the different mawè products showed that *P. kudriavzevii* occurred throughout fermentations in all kinds of mawè (Fig. 4). Group A_1 were only isolated from mawè for come (maize) (site H), whereas group A_2 and A_3 were isolated from all sites. *K. marxianus* were isolated in all kinds of mawè and occurred mostly toward the intermediate stage until the end of the fermentation. *S. cerevisiae* were only isolated in commercial mawè (maize) (site C,D) and mawè for come (maize) (site G,H), with the highest amounts at the end of the fermentation of mawè for come (maize) (Fig. 4d).

During processing, sorghum was only used as raw material for commercial mawè (sorghum) (Fig. 1). In commercial mawè (sorghum) only *P. kudriavzevii* and *K. maxianus* was isolated, hence exhibiting the least diverse yeast microbiota of the four kinds of mawè (Fig. 4a). Mawè for come (maize) had the most differing processing from the three other kinds of mawè, in which the sorted maize were directly soaked in boiled water (Fig. 1). At both processing sites (G,H) *P. kudriavzevii* comprised 100% of the isolated yeasts at 0h of fermentation, which was otherwise only observed for commercial mawè (sorghum) from site B (Fig 4a,d).

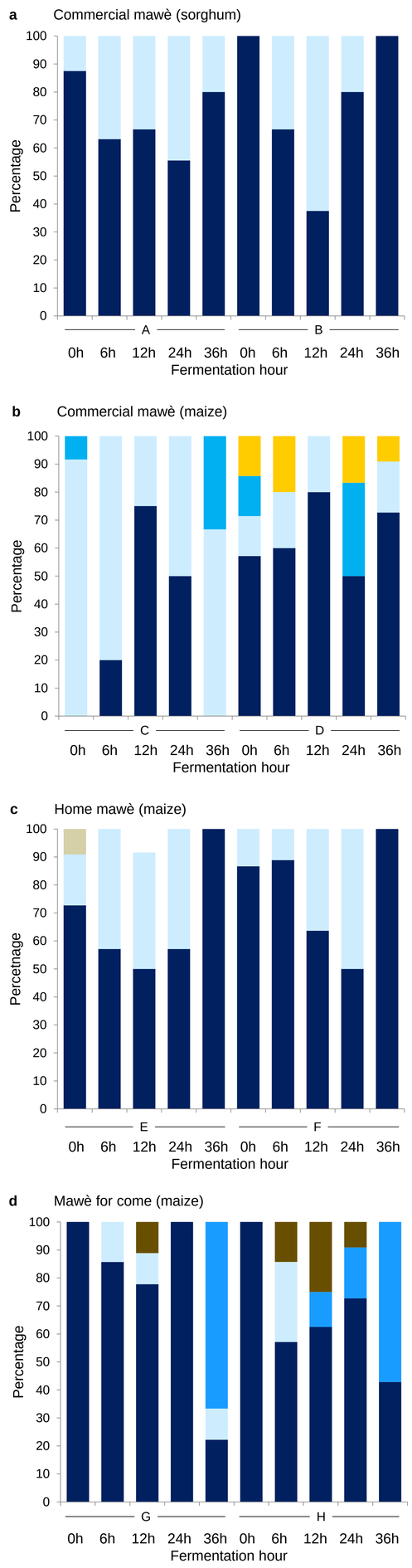

Fig. 4. Relative quantifications of the yeast isolates at 0h, 6h, 12h, 24h, and 36h of fermentation of (a) commercial mawè (sorghum) produced at site A,B; (b) commercial mawè (maize) produced at site C,D; (c) home mawè (maize) produced at site E,F; (d) mawè for come (maize) produced at site G,H. ■ *P. kudriavzevii*, ■ *K. marxianus*, ■ *S. cerevisiae*, ■ *O. polymorpha*, ■ *C. glabrata*, ■ *W. anomalus*.

Conclusion

The predominant yeast species in mawè fermentations were different biotypes of *P. kudriavzevii* (66% of the isolated yeasts) followed by *K. maxianus* (25% of the isolated yeasts). Slight variations were observed in the yeast successions for the different kinds of mawè, which could be due to the differences in raw materials and processing methods used for mawè production.

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