

Control of the Growth of Coliforms and Moulds in Sorghum Malting by Bacterial and Yeast Cultures

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ABSTRACT

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Traditional outdoor floor malted sorghum can be contaminated with high levels of coliform bacteria and moulds that can potentially produce mycotoxins. The effect of steeping with the addition of bacterial and yeast starter cultures on microbial contamination, toxicity and diastatic power (DP) of sorghum malt was investigated. *Lactobacillus plantarum*, *Pediococcus pentosaceus* and *Saccharomyces* species were selected on the basis of inhibition of fungal species using the disc diffusion assay. Steeping the sorghum with the addition of these cultures at 10^7 – 10^8 cfu/mL reduced the level of moulds by one to two log cycles, with the *Saccharomyces* culture appearing to be the more effective and *L. plantarum* the least effective. Coliforms, where present in significant numbers, were reduced to levels of $<10^1$ – 10^2 by steeping with the cultures, with the *P. pentosaceus* culture appearing to be the most effective and *Saccharomyces* the least effective. There were no detectable amounts of mycotoxins and no significant cytotoxicity in the malts in either the controls or the culture-steeped malts. Steeping with the cultures did not affect malt DP. The use of such *P. pentosaceus* and *Saccharomyces* sp. cultures could be an alternative to chemical treatment for the control of microbial contamination in sorghum malt.

Key words: bacteria, moulds, *Pediococcus*, *Saccharomyces*, sorghum malting.

INTRODUCTION

Traditional outdoor floor malted sorghum can be contaminated with high levels of coliform bacteria and moulds that can potentially produce mycotoxins¹⁷. Recently we have shown that these undesirable sorghum malt microflora can be controlled by steeping in dilute NaOH¹⁶. However, a recent development aims to inhibit the growth of unwanted bacteria and moulds by the addition of desirable microbial cultures as natural biocontrol agents in steep¹⁸. Lactic acid bacteria (LAB)¹⁵ and the yeast *Geotrichum candidum*⁴ have been shown to be effective at inhibiting unwanted microorganisms in barley malting.

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The inhibition of coliforms and moulds by LAB is attributed mainly to the low pH, resulting from the production of lactic and other acids^{21,28}. Synthesis of bacteriocins^{3,10,22} and antifungal compounds²⁸ and depletion of nutrients can also play a role²⁰. Of significance with respect to sorghum malting is that many anti-microbial-producing lactic acid bacteria have been isolated from samples of raw sorghum¹². Two of these, which were identified as *Escherichia* species, were found to be bacteriocin producers. With regard to the antifungal activity of LAB, the precise mechanism by which it takes place is apparently difficult to define as it may often be due to a complex interaction between different compounds and the targeted fungus²⁸. Concerning the mechanism of anti-microbial activities by yeast, Boivin and Malanda⁴ reported that when the yeast *G. candidum* was added during steeping of barley grain it inhibited other microorganisms through competition. This mode of inhibition has also been observed when yeasts were used as biocontrol agents of postharvest diseases¹⁴, as the yeasts were able to colonize and survive on fruit surfaces for long periods of time under different conditions and they also used available nutrients to proliferate rapidly, limiting nutrient availability to the pathogens.

In addition to offering the potential to improve the safety and quality of malt, the use of microbial cultures in steeping is claimed to be easy to use, nature-friendly, inexpensive and not lead to the formation of toxic compounds, nor alter the nutritional and palatability properties of the grain product¹⁸.

The objective of this study was to determine whether the addition of microbial cultures could control the growth of coliforms and moulds in sorghum malting.

MATERIALS AND METHODS

Sorghum grain

Two condensed tannin-free red sorghum cultivars, NK 283 and PAN 8546 were used as described in our previous work^{16,17}.

Microorganisms

Initially several different types of microorganisms (two LAB, five *Bacillus* spp. and two *Saccharomyces* spp.) were screened for their antimicrobial properties. The LAB cultures (*Lactobacillus plantarum*, and *Pediococcus pentosaceus*) were selected based on the fact that they or their

related strains have been previously shown by other researchers to inhibit the growth and survival of some fungi^{2,10,25,27}. The *Saccharomyces* spp. and *Bacillus* spp. cultures were selected because of their antifungal activities found during preliminary work in this study. Details of those microorganism, which were found to be effective at controlling microbial growth in laboratory-scale sorghum malting, are given in Table I.

Eight moulds (*Aspergillus flavus*, *Phoma sorghina*, *Curvularia* sp., *Fusarium oxysporum*, *Fusarium chlamydosporum*, *Fusarium verticillioides*, *Penicillium* sp., *Aspergillus fumigatus* and *Alternaria alternata*) were used. These were selected as they were the most dominant moulds identified in sorghum malt¹⁷.

The LAB cultures were grown on De Man, Rogosa and Sharp (MRS) agar at 30°C and stored on MRS plates at 5°C. Liquid suspensions of the cultures were grown in MRS broth at 30°C. The *Saccharomyces* spp. cultures were grown and stored on malt extract broth and/or agar. The *Bacillus* spp. cultures were grown on nutrient agar at 35°C and stored on nutrient agar and/or broth. The mould cultures were cultivated and maintained on potato dextrose agar (PDA) slants in McCartney bottles at 5°C until required. The cultures were sub-cultured at monthly intervals.

Disc diffusion assay

To select cultures that inhibit the growth of moulds and coliforms during sorghum malting, the antifungal activities of the LAB and *Saccharomyces* sp. listed in Table I were determined against the eight moulds by the disc diffusion method^{19,23}. Moulds were incubated for 5 days at 25°C. Mould growth on slants was harvested into sterile saline (9%) containing 0.1% Tween-80 by brushing the slant surface with a sterile loop. The mould suspension was subjected to vigorous agitation using a vortex mixer to break the clumps. The spore count was then determined with the aid of a haemocytometer and the suspension standardised to a final concentration of 10⁷ spores per mL. A 100 µl aliquot of mould spore suspension was transferred to PDA plates and spread uniformly over the agar surface with a sterile bent glass rod. Sterilized filter paper discs (Whatman No. 1, 5 mm diameter) were saturated with 100 µL of each of the test cultures. The discs were allowed to air-dry. The dry discs were placed on inoculated PDA plates. Each culture was tested in duplicate. Each plate also contained one disc with cycloheximide (1 mg/mL in sterile distilled water) as a positive control and another disc with a sterile uninoculated LAB or PDA broth as a negative control. The plates were incubated at 25°C for 24 h. Zones of inhibition indicating antifungal activity formed around each disc, if present, and were measured in mm. Clear zones of more than 0.5 mm were considered as positive for inhibition⁸.

Laboratory scale malting

Samples (150 g) of the two sorghum cultivars were rinsed and then steeped at 25°C for 8 h in 300 mL still tap water (control). The test cultures, including cells and spent media, were added into the 300 mL steep water to make a final concentration of cultures of about 10⁷–10⁸ cfu/mL. Mixed cultures were also investigated using different proportions of the *P. pentosaceus* (L5) and *Saccharomyces* sp. (Y1) cultures. The inoculated grain was steeped as above. After steeping, the grain was rinsed and then germinated at 28°C for 6 days, and then dried at 50°C as described¹⁶.

Analyses

pH. The pH of the steep water, which was drained after the first 8 h of steeping, was determined.

Other analyses. The sorghum grain at 0 h, at 8 h before rinsing, at 8 h after rinsing, on the second and fourth day during malting (green malt) and after six days (dried malt) were assayed for the following as described in our previous work^{16,17}. Microbial population: total aerobic plate counts (APC), LAB, yeasts, moulds and coliforms by standard plating methods, mould identification and identification, enumeration by the direct plating method. Diastatic Power (DP), according the South African Bureau of Standards method, except that water was used as the enzyme extractant instead of peptone solution⁷, aflatoxins by thin layer chromatography (TLC), fumonisins by the VICAM FumonitestTM, deoxynivalenol (DON) and zearalenone by a multi-toxin TLC screening method, cytotoxicity by the MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) cell proliferation assay.

Statistical analysis. Duplicate samples were evaluated during each analysis and the experiments were repeated three times to obtain a total of six observations for each analysis. The data were analysed using the STATGRAPHICS 5.0 program (Statistical Graphics Corporation, Rockville, USA). Analysis of variance (ANOVA) was used at the 0.05 level of significance. Differences between means were determined by the least significant difference method.

RESULTS AND DISCUSSION

Microbial growth inhibition

It was found that of the microorganisms investigated, the two LAB *P. pentosaceus* (L5) and *L. plantarum* (L9) and one *Saccharomyces* sp. (Y1) exhibited antifungal activity and controlled microbial growth during laboratory malting. Table II shows that with the disc diffusion assay *P. pentosaceus* (L5) showed inhibition against five mould species. The *Saccharomyces* sp. (Y1) inhibited four moulds and the *L. plantarum* (L9) three moulds. The inhi-

Table I. Details of those microorganisms found to be effective at controlling microbial growth in laboratory-scale sorghum malting.

Cultures (Code number)	Original culture numbers	Source
<i>Pediococcus pentosaceus</i> (L5)	Culture no. 13	Cottage cheese, University of Pretoria, South Africa
<i>Lactobacillus plantarum</i> (L9)	B 845	Sorghum, CSIR culture collection, Pretoria, South Africa
<i>Saccharomyces</i> sp. (Y1)	Not available	Sorghum malt, University of Pretoria, South Africa

Table II. Antifungal activity (inhibition zones in mm) of the *P. pentosaceus* (L5), *Lactobacillus plantarum* (L9) and the *Saccharomyces* sp. (Y1) cultures tested against different mould cultures using the disc diffusion assay.

	<i>A. flavus</i>	<i>P. sorghina</i>	<i>Curvularia</i> sp.	<i>F. oxysporum</i>	<i>F. chlamydo-</i> <i>sporum</i>	<i>F. verticil-</i> <i>lioides</i>	<i>Penicillium</i> sp.	<i>A. alter-</i> <i>nata</i>	<i>A. fumi-</i> <i>gatus</i>
Positive control	4.0	5.5	5.0	5.0	4.5	4.8	4.0	4.2	4.5
Negative control ¹
L5	...	2.4	1.0	1.4	2.0	2.4
L9	...	1.2	1.5	2.1
Y1	1.7	...	2.0	...	2.9	...	1.9

¹ 1 mg/mL cycloheximide.

Ellipsis points indicate no inhibition.

Table III. Effect of steeping NK 283 and PAN 8546 sorghum grains in different microbial cultures on the total aerobic plate count (APC) during the malting process.

		0 h	8 h before rinse	8 h after rinse	2 days	4 days	Dry malt
NK 283	Control	3.1 × 10 ⁵ a ¹	4.9 × 10 ⁷ a	1.8 × 10 ⁴ d	1.1 × 10 ⁵ cd	2.7 × 10 ⁶ f	1.4 × 10 ⁴ cd
	L5	2.6 × 10 ⁷ bc	1.1 × 10 ⁹ f	4.6 × 10 ⁴ e	1.4 × 10 ⁵ de	5.4 × 10 ⁵ d	2.8 × 10 ⁴ e
	L9	3.9 × 10 ⁷ cd	5.8 × 10 ⁸ de	2.8 × 10 ⁴ d	6.8 × 10 ⁴ bc	3.5 × 10 ⁵ bcd	1.6 × 10 ⁴ d
	Y1	6.1 × 10 ⁵ a	7.1 × 10 ⁷ ab	1.3 × 10 ⁴ bc	7.4 × 10 ³ a	1.9 × 10 ⁴ a	7.3 × 10 ³ abc
PAN 8546	Control	4.2 × 10 ⁴ a	9.8 × 10 ⁵ a	9.0 × 10 ² a	5.5 × 10 ³ a	1.3 × 10 ⁴ a	4.4 × 10 ² a
	L5	2.5 × 10 ⁷ bc	5.4 × 10 ⁸ de	2.7 × 10 ³ a	4.9 × 10 ⁴ ab	2.2 × 10 ⁵ abc	4.3 × 10 ³ ab
	L9	2.0 × 10 ⁷ b	2.6 × 10 ⁸ abc	1.6 × 10 ³ a	2.6 × 10 ⁴ ab	1.1 × 10 ⁵ ab	1.9 × 10 ³ ab
	Y1	1.6 × 10 ⁷ ab	3.3 × 10 ⁸ cbd	1.4 × 10 ³ a	4.2 × 10 ³ a	3.1 × 10 ⁴ a	4.6 × 10 ³ ab

¹ Mean values in the same column are significantly different from each other (p < 0.05).

Table IV. Effect of steeping NK 283 and PAN 8546 sorghum grains in different microbial cultures on the growth of LAB during the malting process.

		0 h	8 h before rinse	8 h after rinse	2 days	4 days	Dry malt
NK 283	Control	2.2 × 10 ³ a ¹	5.2 × 10 ⁵ a	5.0 × 10 ² a	1.2 × 10 ³ a	8.3 × 10 ³ a	3.9 × 10 ² a
	L5	4.5 × 10 ⁷ c	7.5 × 10 ⁸ d	8.3 × 10 ³ a	1.9 × 10 ⁴ a	4.9 × 10 ⁵ a	2.6 × 10 ⁴ a
	L9	2.7 × 10 ⁷ b	3.0 × 10 ⁸ c	5.9 × 10 ³ a	8.3 × 10 ⁴ a	1.7 × 10 ⁶ a	7.8 × 10 ⁴ a
	Y1	5.9 × 10 ³ a	2.8 × 10 ⁷ a	1.4 × 10 ⁶ b	4.5 × 10 ⁷ b	9.0 × 10 ⁷ b	3.7 × 10 ⁶ b
PAN 8546	Control	1.5 × 10 ³ a	1.4 × 10 ⁵ a	1.4 × 10 ² a	4.3 × 10 ³ a	4.7 × 10 ⁴ a	1.4 × 10 ³ a
	L5	4.1 × 10 ⁷ b	9.2 × 10 ⁷ b	9.3 × 10 ³ a	4.4 × 10 ⁴ a	7.3 × 10 ⁵ a	1.8 × 10 ⁴ a
	L9	3.4 × 10 ⁷ b	8.6 × 10 ⁷ b	7.4 × 10 ³ a	4.3 × 10 ⁴ a	6.9 × 10 ⁵ a	1.4 × 10 ⁴ a
	Y1	1.9 × 10 ³ a	8.6 × 10 ⁴ a	1.9 × 10 ² a	6.0 × 10 ³ a	1.5 × 10 ⁴ a	6.9 × 10 ⁵ a

¹ Mean values in the same column are significantly different from each other (p < 0.05).

Table V. Effect of steeping NK 283 and PAN 8546 sorghum grains in different microbial cultures on the growth of yeasts during the malting process.

		0 h	8 h before rinse	8 h after rinse	2 days	4 days	Dry malt
NK 283	Control	8.9 × 10 ³ a ¹	9.9 × 10 ⁴ a	5.5 × 10 ³ d	1.7 × 10 ⁴ d	5.1 × 10 ⁴ de	2.1 × 10 ³ a
	L5	4.3 × 10 ³ a	3.1 × 10 ⁴ a	1.9 × 10 ² a	9.0 × 10 ² a	1.3 × 10 ⁴ ab	5.2 × 10 ² a
	L9	1.5 × 10 ⁴ a	5.7 × 10 ⁴ a	7.0 × 10 ² ab	2.1 × 10 ³ b	3.6 × 10 ⁴ bcd	1.7 × 10 ³ a
	Y1	1.9 × 10 ⁷ b	2.0 × 10 ⁸ c	6.5 × 10 ³ e	3.1 × 10 ⁴ d	1.6 × 10 ⁵ f	2.9 × 10 ⁴ d
PAN 8546	Control	5.4 × 10 ³ a	2.5 × 10 ⁵ a	2.8 × 10 ² b	7.4 × 10 ² a	2.6 × 10 ³ a	3.0 × 10 ² a
	L5	1.8 × 10 ³ a	7.1 × 10 ⁴ a	4.1 × 10 ² a	1.0 × 10 ³ b	8.0 × 10 ³ ab	4.2 × 10 ² a
	L9	2.7 × 10 ³ a	4.8 × 10 ⁴ a	3.3 × 10 ² a	1.6 × 10 ³ b	2.5 × 10 ⁴ abcd	9.1 × 10 ² a
	Y1	7.4 × 10 ⁷ a	3.8 × 10 ⁷ b	3.2 × 10 ³ c	3.3 × 10 ³ bc	8.0 × 10 ⁴ e	7.7 × 10 ² a

¹ Mean values in the same column are significantly different from each other (p < 0.05).

bition of moulds observed with this assay can be attributed to the production lactic acid^{21,28} and to extracellular antimicrobial compounds, as it has been found that some strains of *L. plantarum* and *P. pentosaceus*^{1,9,10,25,27} produce antifungal proteins. However, such activity in *Saccharomyces* is not documented.

The cultures (L5, L9 and Y1) were then tested for their ability to inhibit the growth of moulds and coliforms during malting. Sorghum cultivar NK 283 represented a very poor grain quality, having only 40% germinability and a

higher level of microbial contamination than PAN 8546, which represented a better quality grain with 88% germinability. Steeping conditions were ideal for microbial growth on the sorghum grain as the APC increased from 10⁴–10⁷ cfu/g at the beginning of steeping, to 10⁷–10⁹ cfu/g at the end of the 8 h of steeping, before rinse (Table III). This increase was probably due to the fact that during steeping, nutrients leach from the grains into the steep water and become available for microorganisms²⁴. At the end of steeping, discarding the steep water reduced the

Table VI. Effect of steeping NK 283 and PAN 8546 sorghum grains in different microbial cultures on the growth of moulds during the malting process.

		0 h	8 h before rinse	8 h after rinse	2 days	4 days	Dry malt
NK 283	Control	3.2×10^4 a ¹	3.8×10^5 d	1.4×10^4 bc	5.2×10^4 b	2.1×10^5 d	5.7×10^4 c
	L5	3.6×10^4 a	9.3×10^4 cd	1.8×10^4 bc	9.3×10^3 ab	3.2×10^3 b	9.7×10^2 a
	L9	3.5×10^4 a	1.5×10^5 b	3.0×10^4 c	3.2×10^4 b	1.1×10^4 bc	4.0×10^3 b
	Y1	3.1×10^4 a	7.3×10^5 de	7.2×10^3 b	1.7×10^4 b	5.1×10^3 b	6.8×10^2 a
PAN 8546	Control	2.9×10^3 b	7.2×10^4 bc	1.1×10^3 ab	3.2×10^3 a	1.1×10^4 a	2.7×10^3 b
	L5	1.8×10^3 b	4.5×10^2 a	5.4×10^2 a	2.1×10^3 a	4.0×10^2 ab	8.6×10^2 a
	L9	1.9×10^3 b	1.5×10^3 a	1.1×10^3 ab	3.8×10^3 a	1.9×10^3 ab	8.8×10^2 ab
	Y1	2.6×10^3 b	1.2×10^2 a	3.4×10^2 a	1.7×10^4 b	3.4×10^2 a	1.3×10^2 a

¹ Mean values in the same column are significantly different from each other ($p < 0.05$).

Table VII. Effect of steeping NK 283 and PAN 8546 sorghum grains in different microbial cultures on the growth of coliforms during the malting process.

		0 h	8 h before rinse	8 h after rinse	2 days	4 days	Dry malt
NK 283	Control	8.7×10^3 ba ¹	1.3×10^5 b	1.9×10^2 bc	6.5×10^2 bc	8.2×10^4 d	1.7×10^4 c
	L5	1.3×10^2 a	$<10^1$ a	$<10^1$ a	$<10^1$ a	$<10^1$ a	$<10^1$ a
	L9	2.7×10^3 ab	2.0×10^2 a	2.4×10^1 ab	2.0×10^2 ab	1.2×10^1 a	2.6×10^1 a
	Y1	2.6×10^3 ab	3.1×10^3 a	2.5×10^2 cd	6.0×10^2 bc	1.8×10^2 b	5.2×10^2 b
PAN 8546	Control	2.6×10^2 a	8.9×10^3 a	7.2×10^1 abc	2.5×10^2 abc	7.2×10^2 bc	6.3×10^1 ab
	L5	2.1×10^2 a	$<10^1$	$<10^1$ a	$<10^1$ a	$<10^1$ a	$<10^1$ a
	L9	1.7×10^2 a	$<10^1$ a	$<10^1$ a	$<10^1$ a	$<10^1$ a	$<10^1$ a
	Y1	1.7×10^2 a	1.4×10^2 a	5.0×10^1 ab	1.6×10^2 ab	1.0×10^2 b	8.7×10^1 ab

¹ Mean values in the same column are significantly different from each other ($p < 0.05$).

Table VIII. Incidence of mould species (% grains infected) and diastatic power (SDU/g) of the NK 283 and PAN 8546 malt samples made with L5 and Y1 steeped grains.

		<i>Penicillium</i> sp.	<i>Eurotium</i> sp.	<i>F. chlamydo-sporum</i>	<i>A. alter-nata</i>	<i>P. sor-ghina</i>	<i>F. verticil-lioides</i>	<i>R. oryzae</i>	<i>Mucor</i> sp.	Diastatic power
NK 283	Control	$36 \text{ c}^1 \pm 6^2$	$37 \text{ d} \pm 7$	$56 \text{ d} \pm 6$	$70 \text{ d} \pm 10$	$62 \text{ d} \pm 9$	$88 \text{ c} \pm 6$	$100 \text{ d} \pm 0$	$100 \text{ e} \pm 0$	$8.5 \text{ a} \pm 0$
	L5	$14 \text{ b} \pm 4$	$22 \text{ c} \pm 3$	$28 \text{ c} \pm 4$	$23 \text{ c} \pm 4$	$30 \text{ c} \pm 4$	$40 \text{ b} \pm 2$	$32 \text{ c} \pm 4$	$36 \text{ d} \pm 1$	$9.0 \text{ a} \pm 0$
	Y1	$9 \text{ a} \pm 3$	$7 \text{ ab} \pm 1$	$23 \text{ bc} \pm 2$	$19 \text{ bc} \pm 2$	$25 \text{ bc} \pm 4$	$35 \text{ b} \pm 1$	$26 \text{ bc} \pm 2$	$25 \text{ c} \pm 2$	$8.6 \text{ a} \pm 9$
PAN 8546	Control	$15 \text{ b} \pm 3$	$18 \text{ bc} \pm 2$	$25 \text{ bc} \pm 1$	$6 \text{ ab} \pm 2$	$18 \text{ abc} \pm 2$	$33 \text{ b} \pm 2$	$22 \text{ b} \pm 2$	$21 \text{ c} \pm 2$	$18.5 \text{ b} \pm 0$
	L5	$6 \text{ a} \pm 2$	$10 \text{ ab} \pm 2$	$14 \text{ ab} \pm 1$	$0 \text{ a} \pm 0$	$8 \text{ ab} \pm 2$	$14 \text{ a} \pm 2$	$10 \text{ a} \pm 3$	$8 \text{ b} \pm 1$	$20.0 \text{ b} \pm 0$
	Y1	$4 \text{ a} \pm 1$	$7 \text{ a} \pm 1$	$4 \text{ a} \pm 2$	$0 \text{ a} \pm 0$	$0 \text{ a} \pm 0$	$6 \text{ a} \pm 1$	$4 \text{ a} \pm 1$	$0 \text{ a} \pm 0$	$19.3 \text{ b} \pm 0$

¹ Mean values in the same column are significantly different from each other ($p < 0.05$).

² \pm = Standard deviation.

number of microorganisms, as the microorganisms were washed out. The numbers increased again during the four days of germination and then drying the malt reduced the numbers. All the cultures added increased APC, with the exception of the APC in the Y1 inoculated sample of NK 283, which was lower throughout the malting process. A possible reason for this is that the APC were outgrown by the inoculated yeast. None of the cultures inhibited LAB (Table IV) or yeasts (Table V). In fact the LAB numbers in the L5 and L9 samples were higher than in the Y1 samples at 0 h and throughout the malting process (Table IV). This was because the inoculation was carried out with *L. plantarum* (L5) and *P. pentosaceus* (L9), which are themselves LAB. Similarly inoculation with *Saccharomyces* sp. (Y1) resulted in elevated numbers of yeasts throughout the malting process (Table V). Generally, the cultures caused a significant reduction in moulds of one to two log cycles (Table VI).

Steeping with cultures L5, L9 and Y1 also inhibited coliforms (Table VII). In the NK 283 malt, where the coliforms were present in significant numbers, they were inhibited by three log cycles by the *Lactobacillus* and *Pedio-*

coccus cultures and two log cycles by the *Saccharomyces* culture.

With both sorghum cultivars, addition of the L5 and L9 cultures reduced the pH of the steep water from 6.0–6.4 at the beginning of steeping to 3.9–4.0 at the end, whereas the pHs of the controls were only reduced to pH 4.5–4.8 (Fig. 1). In contrast, the pHs of the controls were also reduced to a small extent, from around 6.0 at the beginning of steeping to 4.5–4.8 at the end of steeping, whereas the pH of the yeast cultures (Y1) were not reduced substantially. The low pH observed with the LAB cultures was due to the production of lactic acid by the cultures and this was probably responsible for the inhibition of the coliforms. Low pH inhibits coliforms and other food-borne contaminants by dissociating their cell membranes²⁶. A study of the *P. pentosaceus* strain used in this investigation showed that it produced the bacteriocin pediocin, and that the pediocin was responsible for its antimicrobial activities against other bacteria, including *Lactococcus lactis*, *Bacillus cereus* and *Listeria monocytogenes*¹¹. However, pediocins are not active against Gram-negative pathogens¹³ and might therefore not have

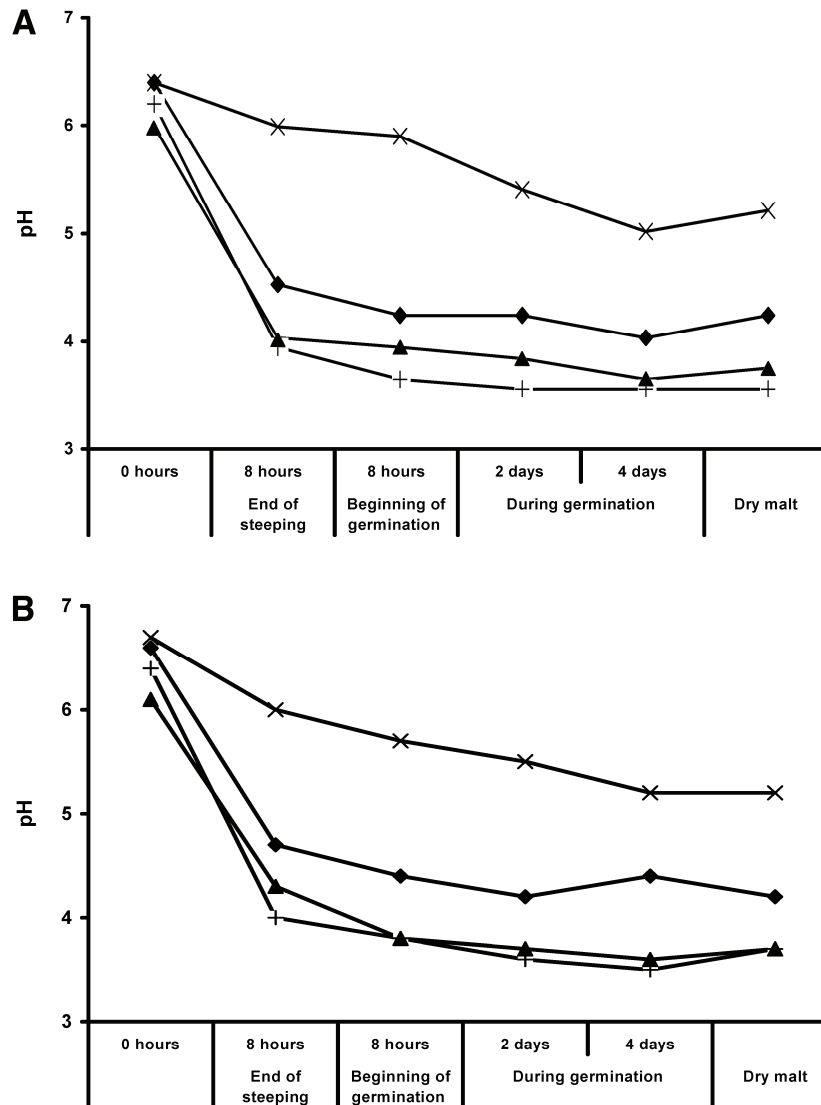


Fig. 1. Effect of steeping the NK 283 (A) and PAN 8546 (B) sorghum grain in different microbial cultures on the pH during the malting process. ♦ = Control; + = L5; ▲ = L9; × = Y1.

Table IX. Effect of steeping the NK 283 and PAN 8546 sorghum grains in the combined microbial cultures on the growth of moulds during the malting process.

		0 h	8 h before rinse	8 h after rinse	2 days	4 days	Dry malt
NK 283	Control	2.0×10^3 b ¹	1.0×10^5 bc	1.3×10^3 b	5.0×10^3 b	2.0×10^3 c	6.3×10^2 a
	50% L5 + 50% Y1	3.2×10^3 b	7.9×10^4 b	4.3×10^3 b	4.0×10^3 b	1.3×10^3 b	4.0×10^2 a
	65% L5 + 35% Y1	3.2×10^3 b	7.9×10^4 b	2.0×10^3 b	3.2×10^3 b	2.0×10^3 b	3.2×10^2 a
	35% L5 + 65% Y1	2.5×10^3 b	1×10^5 bc	6.3×10^3 b	3.2×10^3 b	1.6×10^3 b	3.2×10^2 a
PAN 8546	Control	3.4×10^2 a	1.0×10^4 b	6.0×10^2 a	1.8×10^2 a	7.9×10^2 ab	1.2×10^2 a
	50% L5 + 50% Y1	3.6×10^2 a	5.7×10^3 a	3.4×10^2 a	1.6×10^2 a	1.0×10^3 b	1.0×10^2 a
	65% L5 + 35% Y1	4.0×10^2 a	3.2×10^3 a	4.3×10^2 a	4.5×10^2 a	3.0×10^2 a	1.6×10^2 a
	35% L5 + 65% Y1	3.8×10^2 a	1.5×10^3 a	5.0×10^2 a	3.8×10^2 a	2.5×10^2 a	1.5×10^2 a

¹ Mean values in the same column are significantly different from each other ($p < 0.05$).

been responsible for the inhibition of the coliforms. The production by LAB of low molecular weight compounds (benzoic acid, 5-methyl-2,4-imidazolidinedione, tetrahydro-4-hydroxy-4-methyl-2H-pyran-2-one and 3-(2-methylpropyl)-2,5-piperazinedione) that are active against

Gram-negative bacteria has been reported¹⁹. It is possible that the *P. pentosaceus* strain used in this study produced such antimicrobial compounds. It is also possible that the *P. pentosaceus* strain produced antifungal compounds, as it has been shown that numerous LAB exhibit antifungal

Table X. Effect of steeping the NK 283 (A) and PAN 8546 (B) sorghum grains in the combined microbial cultures on the growth of coliforms during the malting process.

		0 h	8 h before rinse	8 h before rinse	2 days	4 days	Dry malt
NK 283	Control	1.0 × 10 ⁴ b ¹	9.5 × 10 ⁴ b	6.3 × 10 ² ab	1.6 × 10 ³ b	1.9 × 10 ² b	2.2 × 10 ² b
	50% L5 + 50% Y1	3.2 × 10 ² a	6.3 × 10 ³ a	2.3 × 10 ² a	1.2 × 10 ³ b	1 × 10 ³ c	1.6 × 10 ² b
	65% L5 + 35% Y1	4.0 × 10 ² a	3.2 × 10 ³ a	1.5 × 10 ³	6.0 × 10 ² a	3.2 × 10 ² a	1.8 × 10 ² b
	35% L5 + 65% Y1	2.9 × 10 ² a	1.3 × 10 ³ a	5.2 × 10 ² a	4.0 × 10 ² a	2.5 × 10 ² b	1.5 × 10 ² b
PAN 8546	Control	6.3 × 10 ² a	1.0 × 10 ⁴ b	1 × 10 ³ b	6.3 × 10 ³ b	2.0 × 10 ³ c	2.4 × 10 ² b
	50% L5 + 50% Y1	3.8 × 10 ² a	5.9 × 10 ³ a	5.0 × 10 ² a	2.5 × 10 ² a	1 × 10 ² a	6.0 × 10 ¹ ab
	65% L5 + 35% Y1	3.7 × 10 ² a	1.7 × 10 ³ a	1.0 × 10 ² a	1.0 × 10 ² a	2.5 × 10 ¹ a	7.0 × 10 ¹ ab
	35% L5 + 65% Y1	4.0 × 10 ² a	3.2 × 10 ³ a	2.0 × 10 ³ b	1.0 × 10 ³ b	1.0 × 10 ³ c	8.7 × 10 ¹ ab

¹ Mean values in the same column are significantly different from each other ($p < 0.05$).

Table XI. The IC₅₀ levels and concentration of aflatoxins (B₁, B₂, G₁ and G₂), fumonisins (B₁ and B₂), deoxynivalenol (DON) and zearalenone (ZEA) in the NK 283 and PAN 8546 sorghum malts made with L5 and Y1 steeped grains.

	NK 283 and PAN 8546					
	IC ₅₀ ¹ (mg/kg)		Aflatoxins ⁴ (µg/kg)	Fumonisin ³ (µg/g)	DON ⁴ (µg/kg)	ZEA ⁴ (µg/kg)
	NK 283	PAN 8546				
Control	125–250	500 ²	<0.25 ⁵	<3 ¹	<3	<3
L5	250–500	>500	<0.25	<3	<3	<3
Y1	250–500	>500	<0.25	<3	<3	<3

¹ Inhibitory concentration = concentration resulting in 50% inhibition of cells with the MTT assay.

² Maximum concentration.

³ Determined using the VICAM FumonitestTM.

⁴ Determined using TLC.

⁵ Minimum detection limit.

activities and the active factors have been identified¹⁸. The inhibition of coliforms by other bacteria can also be attributable to competition for nutrients¹³.

As stated, production of mould-inhibitory compounds by *Saccharomyces* spp. is not documented. Therefore the antimicrobial effect of the Y1 culture against moulds (Table VI) and coliforms (Table VII) might be attributable to microbial competition, as is the case with *G. candidum* during barley malting⁴. Yeasts are good biocontrol agents because they are capable of utilizing available nutrients to proliferate rapidly, colonize and survive for long periods of time under different conditions and therefore limit nutrient availability to bacteria and moulds¹⁴. The detection of yeasts in higher numbers (Table V) than the moulds (Table VI) and coliforms (Table VII) in the Y1 treatment is an indication that it was able to outcompete these other microorganisms and inhibit their growth.

The malts where the greatest mould inhibition had taken place, i.e. malts made with L5 and Y1 steeped grain, were further analysed to evaluate the extent to which individual mould species were inhibited (Table VIII). It is notable that PAN 8546 was less contaminated with moulds than NK 283. This is again probably related to the fact that PAN 8546 was of a better quality than NK 283 with respect to germinability. Poor quality grain is more susceptible to fungal infections, due to the fact that the available oxygen is not utilised fully by the grain and therefore the fungi utilize it for their own growth³. All the mould species (*Penicillium* sp., *Eurotium* sp., *F. chlamydosporum*, *A. alternata*, *P. sorghina*, *F. verticillioides*, *R. oryzae* and *Mucor* sp.) were significantly reduced by steeping with L5 and Y1. The reduction of most of the moulds (*P. sorghina*, *R. oryzae*, *Mucor* sp., *Eurotium* sp.,

A. alternata and *Penicillium* sp.) was significantly greater with the Y1 steeped malt than in the L5 steeped malt. In fact, with the Y1 treatment, mould contamination of the PAN 8546 malt was negligible. Table VIII also shows that the addition of L5 and Y1 cultures did not increase the DP of the malts, unlike when steeping with dilute alkali¹⁶. However, neither did the cultures inhibit malt amylase activity.

As shown in Table VIII, the Y1 *Saccharomyces* culture was able to eliminate more mould species than the L5 *Pediococcus* culture. However, the L5 culture was able to inhibit coliforms to a greater extent than the Y1 culture (Table VII). Therefore, the two cultures were combined in various ratios and added during steeping with the aim of obtaining synergistic antimicrobial activity. However, there was no inhibition of moulds (Table IX) or coliforms (Table X). The lack of inhibition was probably the result of competition between the combined cultures for nutrients, leading to them not growing optimally, contrary to the situation when they were inoculated individually.

Cytotoxicity and mycotoxins

The malt made from NK 283 had slightly higher cytotoxicity than that from PAN 8546 (Table XI). This could have been the result of toxic products from dead grains, since only 40% of the NK 283 was germinable. Steeping the NK283 grain in the L5 and Y1 cultures slightly reduced the cytotoxicity of the malt. However, the very high IC₅₀ values indicated that all the malts were relatively non-toxic.

Mycotoxins (aflatoxins, fumonisin, DON and ZEA) were not found in the controls or malts steeped with the L5 and Y1 cultures. This is in apparent contradiction with

our earlier work which showed mycotoxins to be present in untreated sorghum malt¹⁷. This difference was probably due to the fact in that the germination temperature was not controlled and the excess water after spraying was not removed from the grain. Moulds produce mycotoxins at elevated moisture content and under stressful environmental conditions, such as fluctuations in temperature or extreme (very cold or very hot) temperatures⁶.

CONCLUSIONS

The use of the *P. pentosaceus* and *Saccharomyces* spp. cultures during steeping has potential as a natural microbial biocontrol method in sorghum malting. These cultures inhibit moulds and coliforms to low levels that should not pose health hazards in the sorghum malt. The use of such cultures could therefore be an alternative to the alkali treatment described previously¹⁶. However, due to the complexity of maintaining and growing up pure cultures, it is probable that this technology would not be appropriate for sorghum maltings with poor scientific infrastructure.

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