Control of microbial proliferation on sorghum during malting

by

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DECLARATION

I hereby declare that the thesis submitted at the University of Pretoria for the award of PhD degree is my work and has not been submitted by me for a degree at any other University or institution of higher education.

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ABSTRACT

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In many African countries, including South Africa, sorghum is malted for the brewing of traditional beer. In South Africa, most sorghum malting is by traditional outdoor floor malting, whereby the sorghum grain is steeped for about 8 hours, left outdoors to germinate in an uncontrolled environment. These malting conditions (wet grain and more or less ambient temperature) encourage microbial proliferation. Microorganisms may themselves negatively impact on the safety of the malts. Of more concern is the proliferation of fungi which can potentially produce highly poisonous mycotoxins in the sorghum malt. Microbial proliferation can also affect the quality of malt, and thereby resulting in undesirable malts. Therefore there is a need for efficient and safe ways to control microbial growth during sorghum malting. The aim of this research was to determine processes to produce sorghum malt that is free of unwanted yeasts, coliforms, moulds and mycotoxins.

The first process investigated involved turning the grains during germination. The second process involved the addition of dilute sodium hydroxide (NaOH)/ caustic soda and calcium hydroxide [($Ca(OH)_2$]/lime during steeping and the third process was by the use of biological control methods which involved inoculation with microbial starter cultures. The effect of the three processes on the levels of moulds, coliforms, mycotoxins (aflatoxins, fumonisins, deoxynivalenol and zearalenone), cytotoxicity, expressed in terms of their IC_{50} (Inhibitory concentration resulting in 50% inhibition of the cleavage activity) and quality in terms of diastatic power (DP) of sorghum malt were investigated.

Turning the sorghum grains during germination did not affect the microbial load of the malt. The total bacterial counts were at high levels of 10^7-10^9 cfu/g, fungi at 10^4-10^6 cfu/g and coliforms at 10^3-10^5 cfu/g. Turned and unturned grains produced malt which showed contamination by about 8 different mould species. Some of these moulds (*Fusarium verticillioides, Phoma sorghina. Aspergillus flavus, Alternaria alternata* and *Penicillium* spp.) are known to produce mycotoxins. Malt samples contained fumonisins, deoxynivalenol and zearalenone at levels of < 0.25-2 µg/g, 15-20 and 10-15 µg/kg, respectively. However, they all had very low cytotoxicity (IC_{50} from 31.2 to > 500 mg/kg). Turning had the negative effect of decreasing the DP of the sorghum malt. The reason that turning did not reduce the microbial load is probably due to the fact that the blending of malt as a result of turning ensured that bacteria and moulds were evenly distributed throughout the malt bed.

Steeping sorghum grains in 0.2% NaOH reduced the level of microbial contamination in the malt. Coliforms and moulds were reduced from 10^4 and 10^5 cfu/g respectively, to levels of 10^2 cfu/g in the malt that do not pose health hazards. The high pH (10-13) that resulted from the addition of NaOH probably caused the inhibition of coliforms and moulds by distorting their cell membranes, destroying the proton gradient of the bacterium cell and thus leading to their death. Steeping in 0.2% NaOH resulted in malts with no detectable amounts of mycotoxins and no indication of cytotoxicity in the sorghum malt. A further advantage was that the DP of the 0.2% NaOH steeped malts was doubled.

The addition of about 10^7-10^8 cfu/ml of *Saccharomyces* spp. and *Pediococcus. pentosaceus* cultures to steep water reduced moulds in the malt from 10^4 cfu/g to 10^2 cfu/g and coliforms from 10^4 cfu/g to 10^2 and $<10^1$ cfu/g, respectively. The antimicrobial activity of the *Saccharomyces* spp. appears to be mainly due to the competition with the other microorganisms. The antimicrobial activity of *P. pentosaceus* is mainly attributed to the low pH. In addition to the low pH, production of CO_2, competition for nutrients and the production of antimicrobial activity could have been responsible for the overall antimicrobial activity of *P. pentosaceus.* Steeping with microbial cultures resulted in malts that contained no traces of mycotoxins and cytotoxicity. The DPs of the sorghum malts were not affected by steeping with microbial cultures.

Turning of grains during germination is not a good method to control microbial load during sorghum malting. The addition of dilute NaOH in steeping water is proposed as a chemical

method for the control of bacterial and fungal contamination during sorghum malting whereas the use of the *Saccharomyces* spp. and *P. pentosaceus* cultures offers a potential alternative as natural, biocontrol agents. However, dilute alkaline steeping is a more favoured method because it is an easier and practical method to put into operation.

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