Effect of Temperature on the Formation and Decomposition of Butan-2-3-dione in Wort Brewed with Sorghum and Barley During Fermentation

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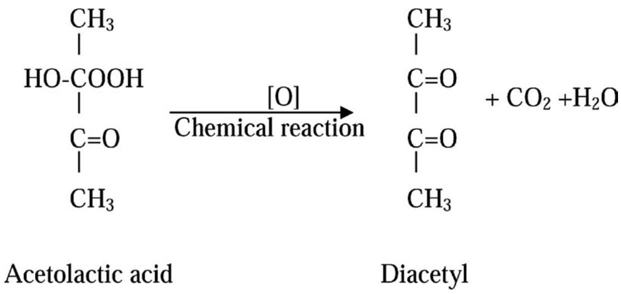
The rate of breakdown of fermentable sugar and the formation/decomposition of butan-2,3-dione (diacetyl) in wort made with unmalted sorghum, malted sorghum, malted barley and sorghum/barley malt adjunct during fermentation was studied as a function of temperature. The rate of fermentation of sugar, formation and decomposition of butan-2,3-dione increases with increasing temperature and is dependent on the nature of the substrate. The decomposition of butan-2,3-dione is faster in wort made with malted sorghum and barley when compared to wort made with unmalted sorghum or sorghum/barley malt adjunct.

Key words:
Fermentable sugar, diacetyl decomposition, temperature.

Introduction

The quality of good lager beer is dictated by the flavor. Different flavour compounds such as 2, 3-pentane dione, acetaldehyde, hydrogen sulphide, and diacetyl (butan-2, 3-dione) are responsible for this, depending on their concentrations in the beer.[1,2] The rate of formation of diacetyl (butane-2, 3-dione) is related to the decomposition of fermentable sugars and the availability of specific amino acids. This is dependent on both temperature and the type of substrate used in fermentation.

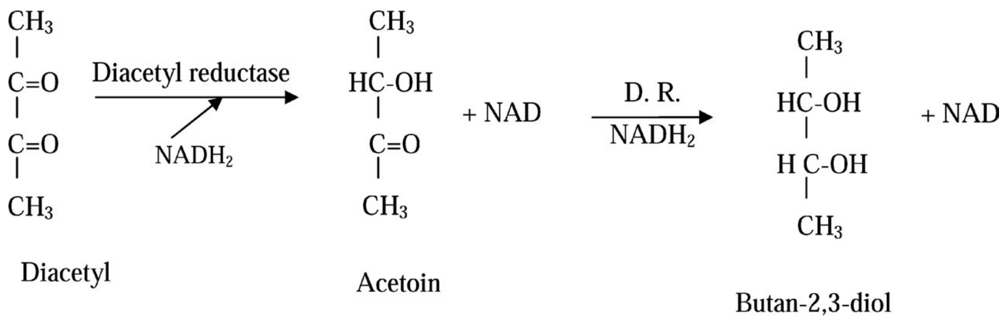
Diacetyl is a diketone responsible for a buttery flavor in beer. It is produced directly by various micro-organisms during brewing. Particularly, it is associated with the infection of lactic acid cocci (*Pediococcus*) and respiratory deficient yeast.[3,4] Normal pitching of yeast produces diacetyl in an unusual indirect way by excreting α-acetolactic acid into the fermenting wort (Scheme 1).



Scheme 1 – *Diacetyl formation scheme*

High levels of assimilable nitrogen in the wort, rapid fermentation and quick removal of yeast from the beer enhance its production. It has been shown that yeast excretes the diacetyl precursor, and also removes the diacetyl by enzymatically reducing it to acetoin and finally to butan-2, 3-diol. (Scheme 2).

The diacetyl is discerned as a butterscotch flavor and aroma at mass concentration range of 0.3



Scheme 2 – *Enzymatic reduction of diacetyl*

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mg L^{-1} – 2.0 mg L^{-1}.[5] The butan-2, 3-diol is almost flavorless. The enzymatic reduction requires yeast in an active metabolic condition and is enhanced by high yeast concentrations, relatively high temperatures and lengthy contact.[5, 6]

Beer can be produced from a host of carbohydrate substances such as cassava, cocoyam, sorghum, barley, maize among others.[7] However, the quality of beer produced is synonymous with acceptability among other factors. Our choice of sorghum (malted and unmalted) for this study, is based on its use as an adjunct to barley in the production of larger beer drinks in Nigeria and the fact that there have been several attempts to use sorghum as a substitute for barley in Nigeria.[8–13] Sorghum (bicolor) commonly called guinea corn, is a widely cultivated cereal. It is a dry fruit with a single seed enclosed in a dry outer covering fused to the seed coat.[14] Sorghum grains have a wide variety of colors, red, black, brown, yellow and white and it can be used for a variety of purposes apart from beer production.[15,16]

Okon and *Uwaifo*[8] have reported that relative similarities exist between malted sorghum and barley. The moisture, cold water extract, kolbach index, total soluble nitrogen parameters have comparable values. However, differences were observed in hot-water extract, 186.3 Lo kg^{-1} for sorghum and 301 Lo kg^{-1} for barley: EBC 53.5 and 76.4 ^{o}WK, fat fractions w = 2.1 and 1.4 %, and diastatic power 40.7 and 90.2 ^{o}L for sorghum and barley, respectively.

Anichie and *Okafor*[17] have shown that malted and unmalted sorghum wort are similar to malted barley. The minor difference lies in the iodine reactions which gave faint yellow tinge in sorghum and no coloration in barley. The faint yellow tinge shown by sorghum indicates that not all the carbohydrate is converted to fermentable sugar and may account for the lower extract recorded for sorghum.

This study is focused on determining the rate of formation/decomposition of diacetyl during fermentation using wort made with malted and unmalted sorghum, malted barley, and malted sorghum as well as barley adjunct.

Experimental methods

Wort samples were prepared using the following cereals

Sample 1: Sorghum

Sample 2: Sorghum malt

Sample 3: Barley malt

Sample 4: Mixture of sorghum and barley malt (sorghum/barley malt adjunct)

Wort of malted sorghum and sorghum/barley malt adjunct were prepared by mashing procedure. Malt of sorghum was prepared by steeping ground sorghum of approximately 4.432 kg in water to a moisture level which allowed it germinate, produce enzymes, and develop an optimal level of brewer's extract. Mashing was carried out by milling 500 g of the wort made with malted and unmalted sorghum. 50 g of the milled samples was weighed into 8 different beakers and 290 ml of distilled water added at 46 °C. 10 ml of $CaCl_2$ was added and 0.2 ml of amylase Ts was also added into the beaker. The whole content of the beaker was thoroughly mixed and heated in a water bath to 70 °C.

The moisture of the grain was determined using a portable moisture analyzer after 34 h of the malting process and was found to be 42 %. The grains were finally steeped in warm water at 40 °C for 2 h (this reduced root growth and brought the moisture to 44.5 %).

During steeping, 1 ml L^{-1} of methanal was added in order to improve germination and prevent the growth of moulds. The grains were allowed to germinate and afterwards, kilned. Kilning was carried out in two stages: the first stage was at 30 °C for about 6 h, the second stage was at 30 °C to 45 °C, then at 60 °C over a period of 8 h. The moisture at this stage was found to be approximately 7.2 %. The mass ratio $\zeta_{sorghum/barley}$ = 70:30 was mashed in the presence of amylase Ts (an industrial enzyme) to release fermentable extract suitable for yeast growth.[17] Mashed samples of adjuncts were filtered and the filtrates were analyzed. The filtered samples were kept at the temperatures of 6 °C, 27 °C and 35 °C. The samples containing wort made with malted / unmalted sorghum, malted barley, and sorghum/barley malt adjunct were pitched with yeast (*saccharomyces calsbergensis*) at the temperature of the experiment. Approximately 30 mls of yeast was added to each sample with a yeast population of $1.3 \cdot 10^6$ cells mL^{-1}.

Determination of the concentration of butan-2, 3-dione (diacetyl)

Diacetyl mass concentration in fermented sugar was determined chemically. Steam-distillate of diacetyl sample was reacted with orthophenyldiamine to form a dimethyl quinoxaline and the absorbance measured at 335 nm.[3,16] The concentration of butan-2, 3-dione in fermented wort was measured spectrophotometrically at 335 nm for 216 h in wort brewed with malted sorghum, unmalted sorghum and malted barley. However, in sorghum/barley malt adjunct the experiment was carried out for 288 h. The concentration of diacetyl was determined using the equation

$$\frac{A_{335} - A_{S}}{A_{S} A_{B}} \cdot 0.625$$

where

A_S is absorbance of standardized diacetyl

A_B is absorbance of the blank

A_{335} is absorbance of the experimental sample at 335 nm

Due to the non availability of pure butan –2,3 – dione (diacetyl), the mass concentration of diacetyl was determined by multiplying the A_{335} with a factor[16] of 2.7.

Results and discussion

Table 1 shows the physical characterization of sorghum and barley filtrates. The faint yellow tinge observed in wort brewed with malted sorghum, unmalted sorghum, and the sorghum/barley malt adjunct suggest that carbohydrates were not fully converted into fermentable sugar in the samples. pH values of the samples show wort brewed with malted barley/sorghum adjunct to be more acidic than the malted barley. A plausible explanation for this observation may be due to the nature of the substrate. Unmalted sorghum wort has the highest pH value of 5.73. The specific gravity of all the samples at 20 °C and their apparent extract were approximately the same.

Extract content and formation/decomposition of butan-2,3-dione as a function of temperature are shown in figures 1 – 4. Plots of mass concentration of butan-2, 3-dione against temperature give curves with maxima (Fig. 1 and 2). From figure 1, relatively high concentration of butan-2,3-dione was obtained with temperature increase. This could have resulted from the yeast growth rate on maltotriose which originates less valine, consequently, α-acetolactate and sequential reduction of diacetyl to acetoin and 2,3 butan diol occur. This must have

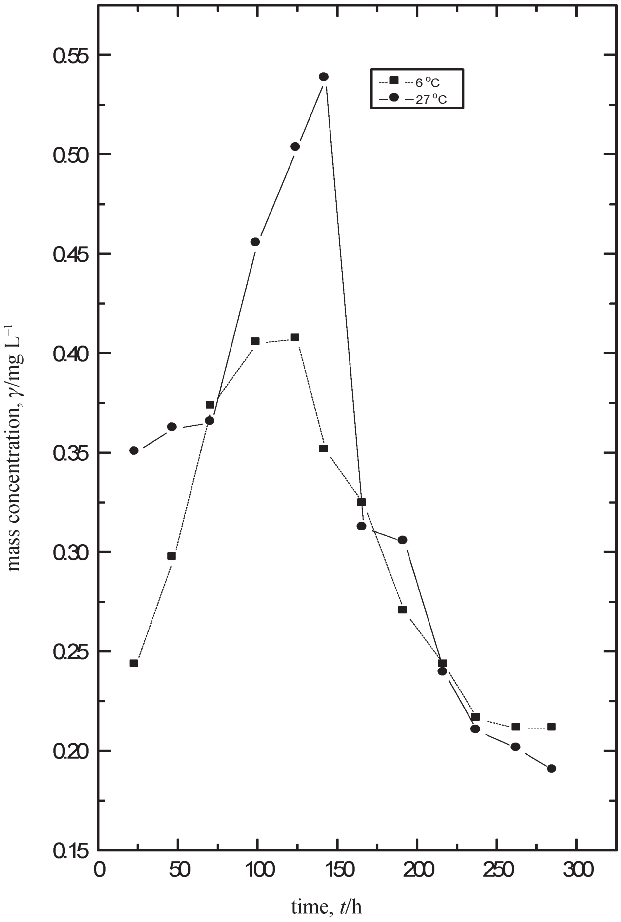
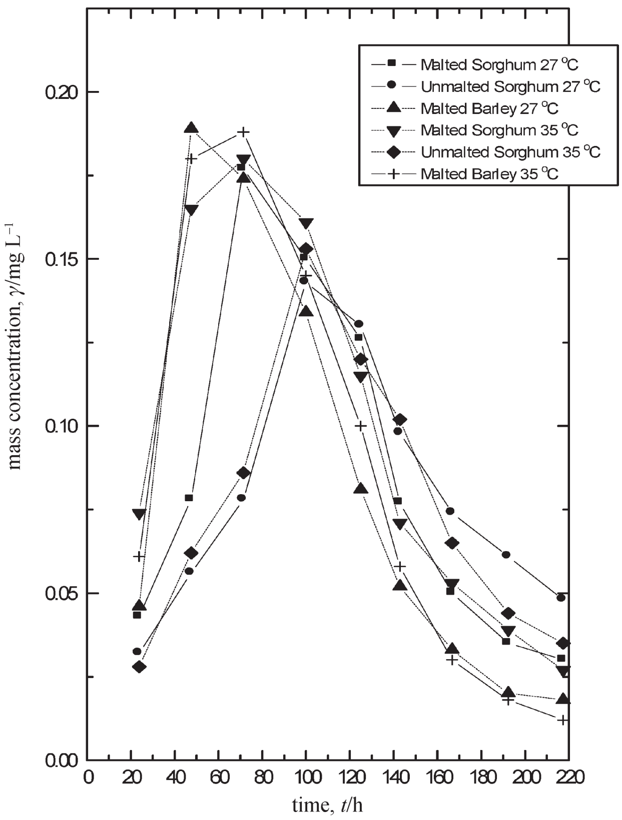


Fig. 1 – *Plot of mass concentration of butan-2,3-one against time in wort made with Sorghum/barley malt adjunct as a function of temperature*

occurred within the first four days of fermentation giving rise to the maxima in figure 2. In all the sample wort, concentration of butan-2,3-dione increases with time (i.e. formation) until a maximum concentration is attained. The rate constant for the formation and decomposition of butan-2,3-dione is taken at tangents of the curves and reported in Table 2. In the table, k_1 represents the rate of formation and k_2 the rate of decomposition of butan-2,3-dione. The

Table 1 – *Aanalysis of filtrates of sorghum and barley*

Quantities	Sorghum wort		Malted barley	Malted barley/sorghum adjunct
	malted	unmalted		
pH	5.62	5.73	5.06	4.70
Iodine reaction (Saccharization)	Faint yellow tinge	Faint yellow tinge	Nil	Faint yellow tinge
Color at 430nm	6.40	5.62	5.87	5.58
Specific gravity 20/20 °C	1.04406	1.04394	1.04415	1.04412
Apparent extract (°P)	10.97	10.94	10.99	11.1



F i g . 2 – *Plot of concentration of butan-2,3-one against time in wort made with malted sorghum, unmalted sorghum, and malted barley as a function of temperature*

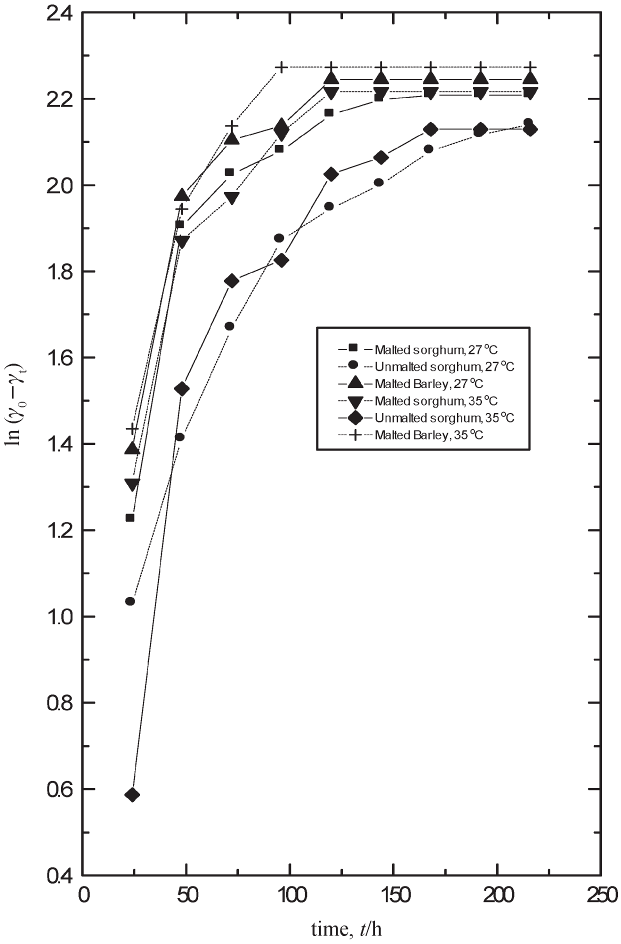
T a b l e 2 – *Temperature dependent rate constants for formation and decomposition of butan-2,3-dione*

Sample	$k_1 \cdot 10^3$/h^{-1}			$k_2 \cdot 10^3$/h^{-1}		
	35 °C	27 °C	6 °C	35 °C	27 °C	6 °C
unmalted sorghum	1.73	1.540	–	0.984	8.541	–
malted sorghum	0.221	0.100	–	1.117	2.792	–
malted barley	4.958	0.967	–	1.108	5.961	–
malted barley/ sorghum adjunct	–	1.938	2.250	–	0.792	1.903

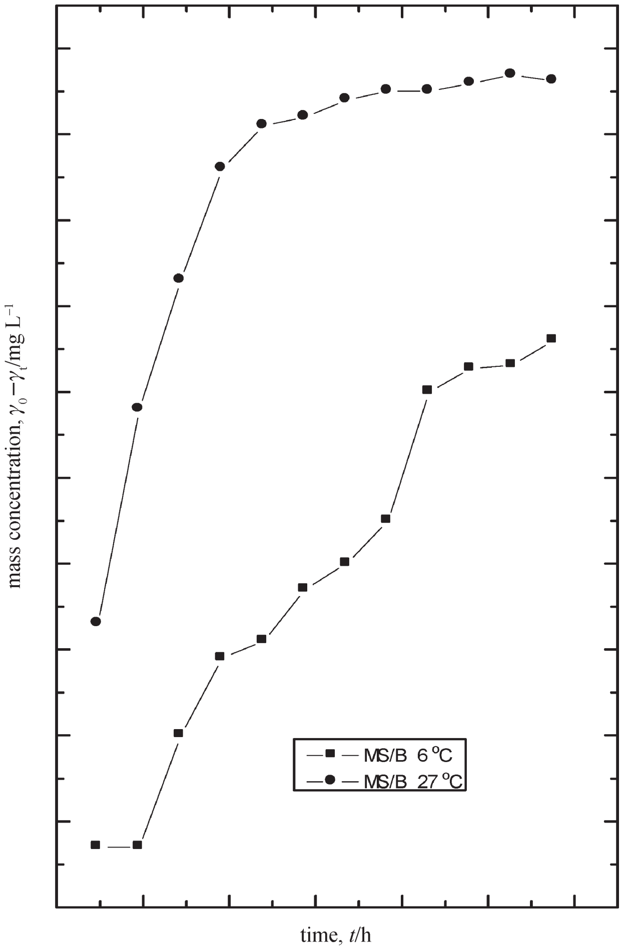
rate coefficients (k_1 and k_2) are of the order 10^{-3} h^{-1} at all the temperatures except at 35 °C for malted sorghum and at 27 °C for malted barley and malted sorghum, where the order is 10^{-4}. The magnitudes of k_1 and k_2 show that the rate of formation is not exactly the same as the rate of decomposition. The results show that the rate of decomposition of butan-2,3-dione is faster than the rate of formation. Also, the rate of decomposition is faster at high temperature, whereas the rate of formation is favored by low temperature.

The results in figure 2 revealed that mass concentration of diacetyl is directly related to the concentration of the extract sugar content. Fig. 3 and 4 show the temperature dependent values obtained for γ_o- γ_t of wort brewed with malted sorghum and barley, unmalted sorghum and sorghum / barley malt adjunct. If the initial concentration of extract sugar content for the substrates at the temperature of the experiment is γ_o and at time t substrate concentration is γ_t , the rate constant and order of the reaction may be obtained from a plot of γ_o- γ_t versus *t*. The rate plots (Fig. 3 and 4) show a pseudo first order reaction rate for the formation of diacetyl prior to the reduction of the diacetyl to acetoin and subsequently 2, 3, butane diol, which is an alcohol. The desired mass concentration in the final product is usually in the range 100 ± 10 mg L^{-1}.[19]

The rate plots (Fig 3 and 4) show a pseudo first order reaction rate for the formation of diacetyl prior to the reduction of the diacetyl to acetoin and subsequently 2,3-butane diol, which is an alcohol. The rate of attenuation (point at which there is no further breakdown of sugar by the yeast) is fa-



F i g . 3 – *Rate equation plot for fermentable sugar in wort made with malted and unmalted sorghum and malted barley*



F i g . 4 – *Variation in mass concentration of fermentable sugar in wort made with sorghum/barley malt adjunct with time*

voured by high temperature. The concentration of extract sugar content decreases with time. At 27 °C, attenuation occurs at 240 h for wort brewed with mixture of sorghum malt and barley malt and at 216 h at 6 °C for the same sample wort, the mass concentration of extract sugar content fermented is larger at 27 °C compared to 6 °C (Fig. 4). The slope of the best fit of the line of regression for the rate of extract sugar content (Fig. 3 and 4) show that the rate coefficients is in the order of 10^{-3} h^{-1}. The wort made with malted sorghum, unmalted sorghum and malted barley is observed to attenuate at 120 h, but with different concentration of extract sugar content, the reaction also gives a first order at the initial time before tending to zero order after 120 h.

Conclusion

Relatively lower wort is extractable from sorghum compared to barley. The rate of formation of butan-2,3-dione is dependent on the breakdown of extract content and both are temperature dependent. While the rate of decomposition of diacetyl is directly proportional to temperature, the rate of its formation showed indirect proportionality with temperature.

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List of symbols

A – absorbance, nm
k – rate coefficient, h^{-1}
m – mass, g
t – time, h
T – temperature, °C
w – mass fraction, %
γ – mass concentration, mg L^{-1}
ζ – mass ratio, m_{sorg}/m_{barl}

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