Evaluation of Three Methods of Application of Malt Crude Enzyme Extracts from Three Cereals in Ghana on Maltose Syrup Production from Cassava Starch

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Abstract - In this study two forms of Crude Enzymes from Malts (CEM): (1). Crude Enzyme Solution (CES) and (2). Homogenised Malt in Cheesecloth Bags (HMCB), from Obaatanpa maize (OM), Sorghum bicolor (SB) and Proso millet (PM) malts were compared to the standard application of Homogenised Malts (HM) on Cassava starch for maltose syrup production. Yields of Sweet Juices and maltose syrups were significantly different ($p \leq 0.05$) among the CEM's and was in the order $CEM_{OM} > CEM_{SB} > CEM_{PM}$. Yields of Sweet Juices and maltose syrups among the CEM's were significantly different ($p \leq 0.05$) and in the order CES > HMCB > HM. DE of maltose syrups depended on the cereal used for the CEM and on the mode of application. Percent reducing sugar and pH of syrups depended on the cereal used for the CEM but was independent of the mode of application. The percent sulphated ash was independent of the cereal used for the CEM and mode of application. The use of CES's instead of HM's for the hydrolysis of Cassava starch significantly ($p \leq 0.05$) increased the yields and DE's of maltose syrups.

Keywords: Cassava starch, Maize, Sorghum, Millet, Malt, Crude Enzymes, Maltose Syrup

I. INTRODUCTION

About forty countries in Africa produce approximately 50% of cassava globally and it is estimated that cassava production in sub-Sahara Africa would account for over 60% of global production by 2020. For the farmers in Africa, the cassava crop provides both food security and a source of income (FAO and IFAD, 2005).

The income obtained by farmers from cassava production is very low because very little value is added through processing to the harvested crop. In Ghana cassava farmers receive low monetary returns from the production of the crop because cassava is a low value product. However, there is great potential for the use of cassava to increase the income of farmers, help reduce poverty, and provide greater food security. (FAO and IFAD, 2005). The economic benefits of cassava can be increased through further processing. This is the case in China where for example, the price of High-Purity Maltose Syrup is five times the price of the cassava from which it is produced (Shuren, n.d.).

Presently, China produces several types of maltose syrups containing varying maltose contents ranging from <50% in ordinary maltose syrup, 50 – 75% High-Maltose syrup, 75 – 95% in Super-High Maltose syrup, and >99% in High-Purity Maltose (Shuren, n.d.).

There is an artisanal process in Indonesia which uses cassava starch and amylase enzymes from rice seedlings for the production of maltose syrup. However, starch from other sources and amylase from other germinated cereals could be used in the process (Quynh and Cecil, 1996).

Ameko et al (2013) adapted the artisanal process described by Quynh and Cecil (1996) to produce maltose syrup from fresh cassava starch (Esiaba var.) by using finely homogenised Obaatanpa maize malt as the source of crude amylase enzymes. Ameko et al (2013) added the homogenised maize malt directly to a solution of gelatinised Cassava starch.

The Gelatinised Starch Solution was converted to Liquefied Starch Solution (LSS) through partial hydrolysis of the starch by alpha amylase enzymes. The LSS contained maltodextrins composed mainly of oligosaccharides and dextrins (Maps Enzymes, 2010). The LSS was hydrolysed further by beta amylase to a Sweet Saccharified Starch Solution (SSSS) containing maltose and glucose units (Sigma Process Technologies, 2014). The SSSS was filtered through cheesecloth to yield a Sweet Juice (SJ) which was then concentrated by evaporation through boiling to form Maltose Syrup.

The yield of maltose syrup obtained by Ameko et al (2013) was 23.59% (ml/g wet starch). The low yield was attributed to the trapping of a large portion of the SSSS in a viscous slurry formed from fine particles of corn flour that were released from the seed part of the homogenised maize into the gelatinised starch solution at the beginning of the process. The SSSS was bound so tightly within the slurry that only a little portion was released as Sweet Juice.

In addition, the fine particles of corn flour blocked the pores of the cheesecloth and reduced the amount of SSSS draining through the cheesecloth during filtration. Furthermore, the presence of the fine materials in the Sweet Juice during

evaporation darkened the final product (Quynh and Cecil, 1996).

The SSSS trapped within the slurry represented loss of potential maltose syrup. This would translate into substantial losses in earnings in a commercial venture and would affect the viability of any artisanal maltose production project. The price of the maltose syrup would have to be increased in order to recover cost and this would be passed onto the customer. This would affect the profitability of the venture, and the higher prices would impact negatively on consumer patronage of the product. More time and energy would also be spent during production trying to obtain more Sweet Juice from the SSSS.

A more suitable method was therefore needed for applying the malt crude enzymes to the gelatinised starch solution so that there would be limited or no introduction of fine particles of corn flour from the malt crude enzymes into the reaction mixture.

In a procedure described by Olempska - Beer (2007) for the production of Isoamylase from Pseudomonas amyloderamosa, the fermentation broth containing the Isoamylase extracellular enzyme was filtered to remove cellular debris. The enzyme solution was then concentrated and purified.

According to Ekunsaumi (2002) a crude amylase solution can be produced in the laboratory from fungi by removing the fungal mycelium from the enzyme production medium by filtration through filter paper.

Siddiqui et al., (2012) prepared a crude enzyme solution from solid fermented media of decomposing orange peels by extraction with distilled water. The fermented media was vigorously mixed with distilled water and then filtered through cheesecloth. Cheesecloth is also used for filtering wine prior to wine testing (Cheesecloth Canada, 2010).

In an earlier study (Ameko et al., 2013), crude enzymes in the form of finely homogenised Obaatanpa maize malt was added directly to a solution of gelatinised Cassava (Esiaba var.) starch to produce maltose syrup.

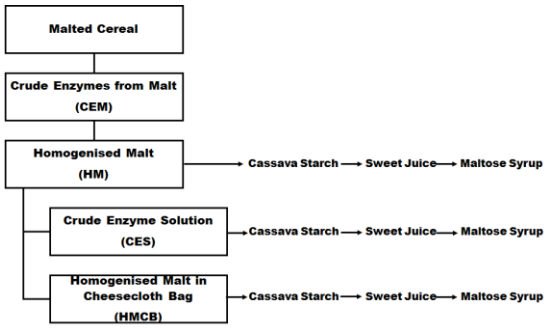

Fig. 1. Forms of Crude Enzymes from Malted Cereals and Modes of Application on Cassava Starch for Maltose Syrup Production

In this study, homogenised malts from Obaatanpa maize, Sorghum bicolor and Proso millet were used to prepare two other forms of crude enzyme extracts (Fig. 1):

- Homogenised malts contained in cheesecloth bags
- Crude enzyme solutions

The two were then compared to the homogenised malts for their abilities to produce maltose syrup from cassava starch.

II. Materials and Methods

A. Samples

The cereal samples used in this study were Obaatanpa Maize, Sorghum bicolor, and Proso Millet coded as OM, SB and PM respectively for this study. Starch was extracted from fresh cassava (Esiaba var.) by the method of Ashveen et al. (2008).

B. Germination of Cereals

The samples of cereals were screened for their suitability for malting by determining their germinative capacities by the method of Hudec and Muchova (2008). Cereal samples with germinative capacities >95% were selected for production of malt for production of maltose syrups (Quynh and Cecil, 1996).

Germination of cereals into malt was done according to the method of Quynh and Cecil (1996) for the minimum number of days required for optimum amylase enzyme activity in the malt (Ameko et al., 2013).

C. Preparation of Crude Enzymes from Malted Cereals

Sprouted seeds of each cereal type were finely homogenised with a mortar and pestle and the homogenised malt divided into twelve equal parts by weight.

Four parts by weight each were used to prepare three different types of crude enzyme applications.

- Homogenised Malts in Cheesecloth-Bags (HMCB)
- Crude Enzyme Solutions (CES)
- Homogenised Malts (HM)

1) Preparation of Homogenised Malts in Cheesecloth Bags (HMCB): The first four parts by weight of homogenised malts were enclosed in four separate bags made from Grade #50 cheesecloth (Cheesecloth Canada, 2010) and these were used as the source of crude amylase enzyme by immersing the cheesecloth bags and their contents in the reaction mixtures.

2) Preparation of Crude Enzyme Solutions (CES): The second four parts by weight of homogenised malts were used to prepare crude enzyme solutions by adding two parts by volume of distilled water to the homogenised malt in a bowl. The mixture was stirred vigorously, left undisturbed for one hour, and the supernatant decanted off the mash into a beaker. More water was added to the mash which was then strained through a cheesecloth into the beaker. The residue in the cheesecloth was washed repeatedly with distilled water into the beaker until the filtrate run clear. The contents of the beaker was filtered again through two layers of cheesecloth to yield a crude enzyme solution.

3) Preparation of Homogenised Malts (HM): The last four parts by weight of homogenised malts were used for the control experiment.

D. Production of Maltose Syrups

1) Treatment 1- Homogenised Malts (HM): This was the control treatment. Maltose syrups were produce according to the method of Quynh and Cecil (1996). The Homogenised Malts served as crude enzyme sources.

The following materials were mixed into a slurry ($Slurry_1$): 1 part by weight homogenised malt, 10 parts by weight wet starch, and 3.5 parts by volume cold water.

A second slurry ($Slurry_2$) was also formed from 1 part by weight homogenised malt, 10 parts by weight wet starch, and 3.5 parts by volume cold water.

Boiling water (30 parts by volume) was added to $Slurry_1$ to form Gelatinised Starch Solution and this was stirred until it thinned out into a free running only a little thicker than water. The LSS was then boiled without burning and then added to $Slurry_2$. The combined slurries ($Slurry_{12}$) were mixed very well and incubated for 6 hours at 62 – 65^{o}C.

An extra 2 parts by weight homogenised malt was mixed thoroughly with $Slurry_{12}$ which was then incubated further at 62 – 65^{o}C. Frequent tests were done with iodine solution for the presence of starch in $Slurry_{12}$ until the results turned negative and a Sweet Saccharified Starch Solution (SSSS) was obtained. The SSSS was filtered through cheesecloth to yield a Sweet Juice (SJ) which was then evaporated through boiling to approximately one-fourth its volume to form maltose syrup.

2) Treatment 2 - Homogenised Malts in Cheesecloth-Bags (HMCB): Maltose syrup was produce according to a slight modification of the method of Quynh and Cecil (1996). In the modification, the Homogenised Malts in Cheesecloth-Bags were used in place of homogenised malt as the source of crude amylase enzymes by immersing the cheesecloth bags and their contents in the reaction mixtures.

3) Treatment 3 - Crude Enzyme Solutions (CES): Maltose syrup was produce according to a slight modification of the method of Quynh and Cecil (1996). In the modification, the Crude Enzyme Solutions were used as sources of crude amylase enzymes in place of the homogenised malts. Part of the cold water used to prepare the slurries was used to prepare the crude enzyme solution.

E. Analysis of Maltose Syrups

The yields of Sweet Juices were determined by measuring the respective volumes with a measuring cylinder.

Moisture content of the maltose syrups were determined by the AOAC method (AOAC, 2000) using a hot air oven at 105^{O}C. The moisture contents of the various syrups were then adjusted to 20% (Dziedzoave, 2004) before further analysis on the other parameters.

The yields of syrups were determined by measuring the respective volumes with a graduated measuring cylinder. The percent yield of maltose syrup was then calculated as:

$$= \frac{\text{Volume of syrup(ml)}}{\text{Weight of wet starch(g)}} \text{x}100 \quad (1)$$

The pH of maltose syrup was determined with a pH meter (Cyberscan PC 6000) according to the method of the International Starch Institute (1997).

The percent reducing sugars was determined after which the Dextrose Equivalence (DE) was calculated (Corn Refiners Association, 1999).

Sulphated ash was determined according to the method of the International Starch Institute (1997).

III. Results and Discussion

The germinative capacities of the cereal samples were Obaatanpa Maize (OM) 96.0% ± 1.1%, Sorghum bicolor (SB) 97.4% ± 1.3%, and Proso millet (PM) 96.4 ± 1.1%. In the artisanal maltose process in Vietnam, germinative capacities of at least 90% but preferably > 95% are required for cereals used in the process (Quynh and Cecil, 1996).

Maximum amylase activities were observed in the germinating seedlings on day 6, 4 and 9 of germination for SB, OM and PM respectively. Gimbi and Kitabatake (2002) observed highest amylase activities in the malt of African finger millet (Eleusine coracana (L) Gaertener) on day 5 – 9 of germination, whilst Ameko et al. (2013) observed maximum amylase activity in OM on day 4 – 5 of germination.

The moisture content of the wet starch samples was 13.2% ± 0.34%. The moisture content of the starch determines the amount of water added to form the slurries. In the artisanal process, the amount of water used to form the slurries with dry starch is approximately twice that for wet starch (Quynh and Cecil, 1996).

A. Yields of Sweet Juices and Maltose Syrups

The different modes of applications of the crude enzymes gave significantly different ($p \leq 0.05$) yields of Sweet Juices, with the Crude Enzyme Solution (CES) giving the highest yields of Sweet Juices (908 ml – 1760 ml), followed by the Homogenised Malt in Cheesecloth Bag (HMCB) treatment with 816 ml – 1224 ml Sweet Juices, and 720 ml – 1060 ml Sweet Juices from the Homogenised Malt (HM) treatments (Fig. 1).

The various malt crude enzymes produced significantly different ($p \leq 0.05$) yields of Sweet Juices with the highest yields produced by the OM crude enzymes, followed by the SB crude enzymes, and then the PM crude enzymes respectively (Fig. 2).

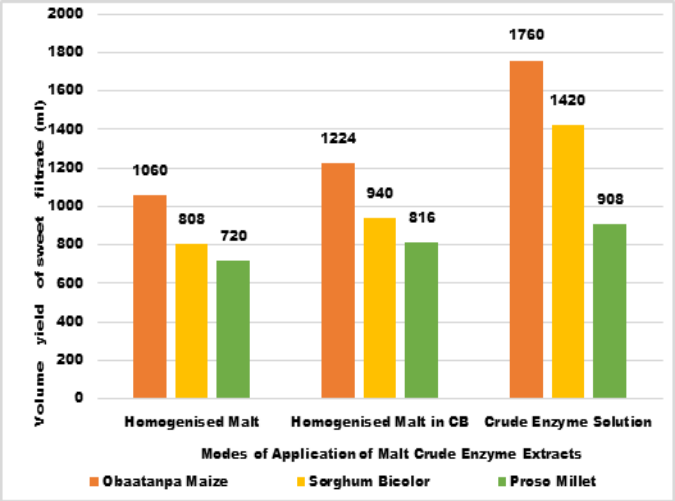

Fig. 2. Three Modes of Applications of Malt Crude Enzyme Extracts from Three Cereals on the Yields (Ml) Of Sweet Juices from Cassava (Esiaba Var.) Starch Prior to Concentration to Maltose Syrup by Evaporation from Boiling

In the preparation of the CES, the solid debris and fine particles of flour released from the malts were retained by the cheesecloth during filtration while the soluble enzymes in solution passed through the cheesecloth to yield a debris free CES. The process of filtration ensured that the CES was free of fine particles of cereal flour (Heidcamp, n.d.).

Fig. 3. i. $HMCB_{OM}$ ii. CES_{OM} iii. HM_{OM} Reaction mixtures of

i. Gelatinised cassava starch and Obaatanpa maize HMCB
ii. Gelatinised cassava starch and Obaatanpa maize CES
iii. Gelatinised cassava starch and Obaatanpa maize HM

The reaction mixture from the HMCB treatment was devoid of plant debris, but had a very thin layer of sediment of fine starch particles from the malted cereals (Fig. 3 i.). The addition of the CES to gelatinised starch solution yielded a debris - free reaction mixture (Fig. 3 ii.). On the other hand, the addition of HM to gelatinised starch solution resulted in the formation of an upper layer of clear hydrolysate and a thick layer of sediment consisting of plant debris and fine starch particles from the malted cereals (Fig. 3 iii.).

During filtration all the Sweet Saccharified Starch Solution (SSSS) from the CES treatment drained through the cheesecloth filter to yield Sweet Juice. During filtration of the SSSS from the HMCB treatment, a significant amount of the SSSS was trapped within the homogenised malt within the cheesecloth bag. Any attempt to squeeze the bag to release the trapped SSSS from the bag resulted in the expulsion of fine starch particles from within the bag through the pores of the cheesecloth into the Sweet Juice thereby contaminating the Sweet Juice. The only way to clarify the Sweet Juice was to allow the particles to settle at the bottom of the Juice and then decant off the clear portion of the Sweet Juice. However, this resulted in the loss of some amount of Sweet Juice which remained trapped within the viscous slurry.

In the HM treatment, the filtration of the SSSS was significantly impeded by the large amount of viscous slurry formed from the plant debris and fine starch particles from the malted cereals.

The different modes of applications of the crude enzymes gave significantly different ($p \leq 0.05$) volumes of maltose syrups, with the Crude Enzyme Solution (CES) giving the highest volumes of 192 ml – 424 ml, followed by the Homogenised Malt in Cheesecloth Bag (HMCB) treatment with 173 ml – 304 ml, and 149 ml – 261 ml maltose syrups from the Homogenised Malt (HM) treatments (Fig. 4).

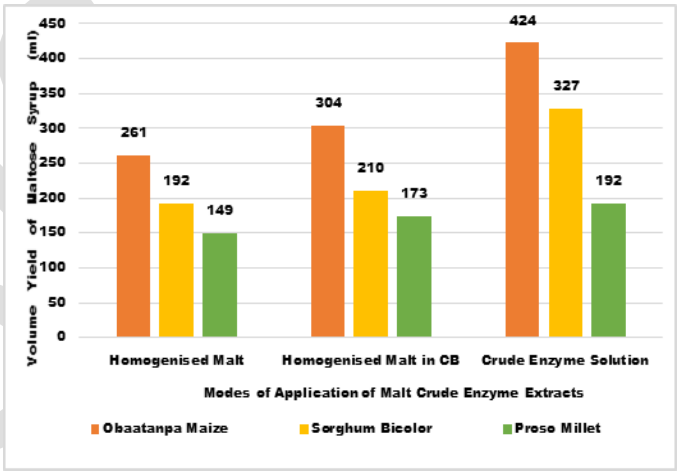

Fig. 4. Three Modes of Applications of Malt Crude Enzyme Extracts from Three Cereals on the Volume (Ml) Of Maltose Syrups from Cassava (Esiaba Var.) Starch

The various crude enzyme sources produced significantly different ($p \leq 0.05$) volumes of maltose syrups with the highest volumes produced by the OM crude enzymes, followed by the SB crude enzymes, and then the PM crude enzymes respectively (Fig. 4).

Compared to the HM control, the CES treatments improved the volumes of maltose syrup more than the HMCB treatments did. For example, the improvement in volume by the CES_{OM} was 62.4% compared to 16.5% by the $HMCB_{OM}$ (Fig. 5). The same trend was observed for SB and PM.

The order of improvements in volumes by the CES's was $CES_{SB} > CES_{OM} > CES_{PM}$, and that for the HMCB's was $HMCB_{OM} > HMCB_{PM} > HMCB_{SB}$.

The percentage yields (v/w) of maltose syrup produced by OM, SB and PM were significantly different ($p \leq 0.05$). The percentage yields (v/w) of the syrup by the HM's, HMCB's and CES's were also significantly different ($p \leq 0.05$).

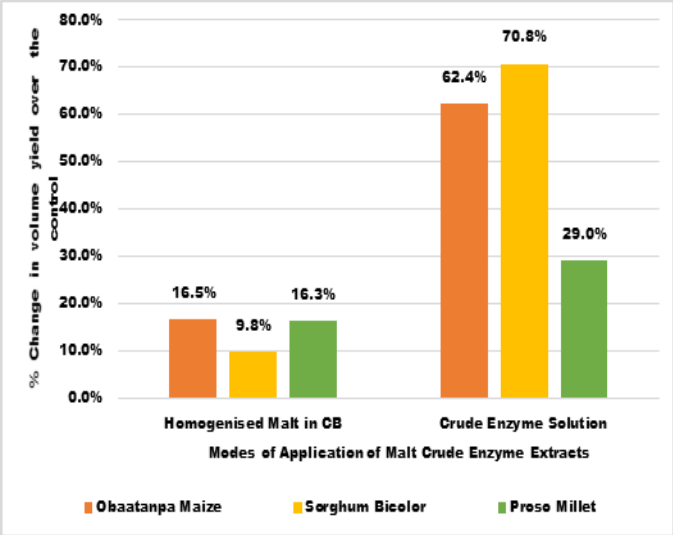

Fig. 5. Percent Changes in Volume of Maltose Syrup When Malt Crude Enzyme Extracts from Three Cereals were applied by Two Different Methods on Cassava (Esiaba Var.) Starch. The Yields were Compared to that from the Control (HM) Treatment

Irrespective of the method of application of the crude enzyme extract on gelatinised starch solution, the OM applications gave the highest percentage yields (v/w) of maltose syrup, followed by the SB and PM applications respectively (Fig. 6).

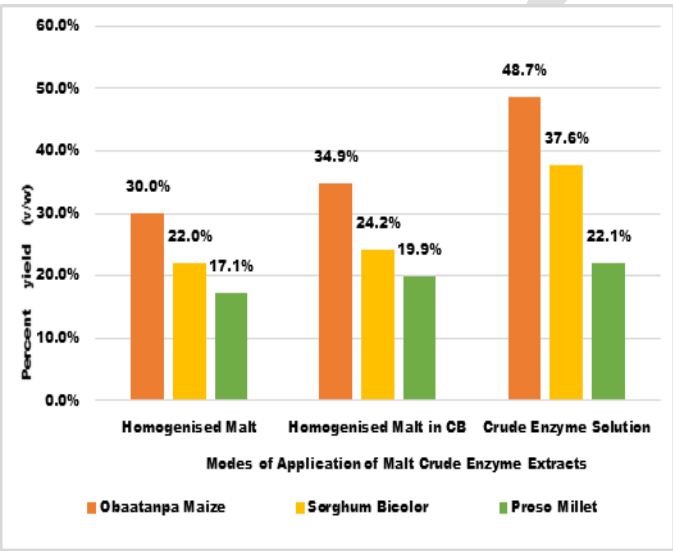

Fig. 6. Three Modes of Applications of Malt Crude Enzyme Extracts from Three Cereals on the Percent Yields (V/W) of Maltose Syrups from Cassava (Esiaba Var.) Starch

The percent yield by the HM_{OM} in this study was 30.0% and this was comparable to the 23.59% yield obtained by Ameko et al (2013). The yields by the $HMCB_{OM}$ and CES_{OM} were 34.9% and 48.7% respectively (Fig. 6). Similar results were obtained for SB and PM.

B. Dextrose Equivalences

The DE's from the different modes of crude enzyme application were significantly different ($p \leq 0.05$), with the CES treatments giving the highest DE compared to the HMCB and HM treatments respectively.

DE's obtained from the application of crude enzyme extracts from the various malts were also significantly differences ($p \leq 0.05$).

The malts arranged in the order of DE values of maltose syrups obtained by the application of their respective crude enzyme extracts was MP > SB > OM, for both HM and HMCB. However, for the CES the order was OM > MP > SB (Fig. 7).

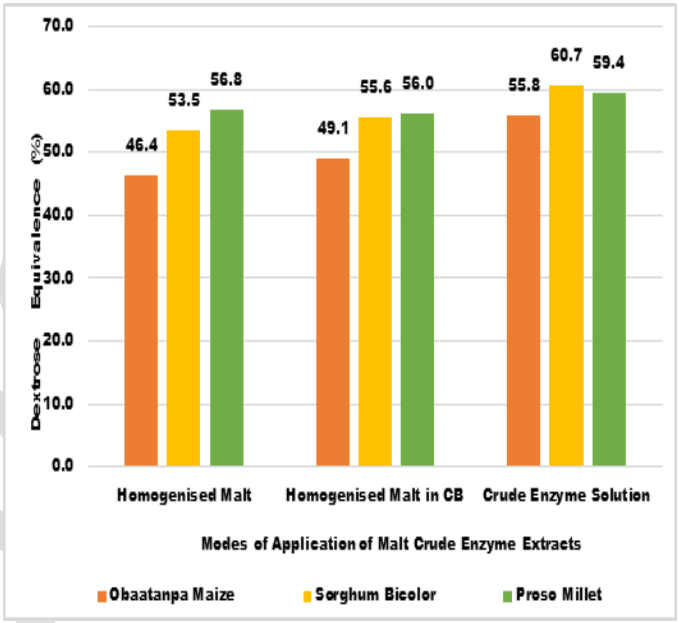

Fig. 7. Three Modes of Applications of Malt Crude Enzyme Extracts from Three Cereals on the DE of Maltose Syrups from Cassava (Esiaba Var.) Starch

DE for syrups from the control HM treatment ranged between 46.39% to 56.84%; 49.10% to 56.04% from the HMCB treatments and; 55.76% to 60.71% from the CES treatments.

The DE value of 46.39% obtained in this study from the HM_{OM} treatment was comparable to the 45% DE of maltose syrup from high quality cassava starch by rice malt crude enzyme extract (Dziedzoave, et al. 2004), the 40% obtained by Ameko et al (2013) for maltose syrup from cassava starch by OM malt crude enzyme extract and . The DE values (46.39% - 60.71%) for cassava starch maltose syrup obtained in this study from the OM, SB and PM malts agreed with the 55% - 62% DE of Arasaratnam et al. (1998) for corn starch sugar syrups, and were higher than the 36% - 42% DE for banana starch glucose syrups (Bello-Perez et al., 2002).

Zainab et al, (2011) used purified Amyloglucosidase to obtain glucose syrup yields and DE's of 86.71% (DE 73.50%), 65.94% (DE 65.66%) and 64.71% (DE 65.66%) for maize starch, millet starch and sorghum starch respectively.

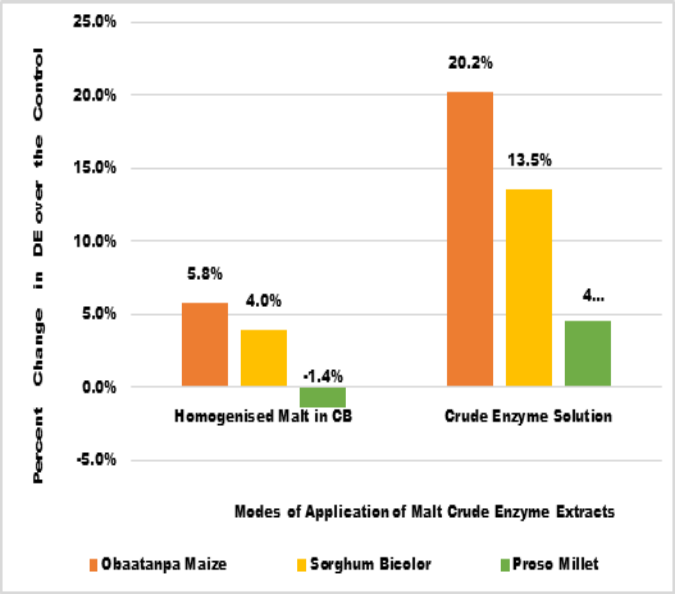

Fig. 8. Percent Changes in DE of Maltose Syrup When Malt Crude Enzyme Extracts from Three Cereals were applied by Two Different Methods on Cassava (Esiaba Var.) Starch. The DE's were Compared to those from the Control (HM) Treatments

The CES_{OM} gave the highest increase (20.2%) in DE over the control, followed by the CES_{SB} and CES_{PM}. For all three malts, the CES treatments gave the highest increase in DE over the control than did the HMCB treatments (Fig. 8).

According to Soto et al. (2012) in the presence of excess amylase enzymes the DE from starch hydrolysis is proportional to the hydrolysis time. The rate of enzymatic hydrolysis of starch depends on the viscosity of the starch solution, and this in turn depends on the amylose – amylopectin ratio of the starch.

The hydrolysis time is also dependent on type of starch and the source of amylase enzymes. For example, the hydrolysis time for the reaction mixture of rice starch – A. oryzae α-amylase is less than for corn starch – A. oryzae α-amylase or potato starch – A. oryzae α-amylase (Soto et al., 2012).

C. pH

The pH of maltose syrups from the PM, OM, and SB treatments in this study were 3.5 – 4.3, 4.6 – 5.3, and 5.1 – 6.3 respectively (Fig. 9), compared to the pH of 4.60 – 5.30 obtained by Ameko et al (2013) for maltose syrup from cassava starch by OM malt crude enzyme extract, and pH 5.5 - 6.5 for glucose syrup from the hydrolysis of high quality cassava flour (HQCF) by rice malts (Dziedzoave et al., 2004).

The crude enzymes from the various malts (OM, SB and PM) produced maltose syrups with significantly different ($p \leq 0.05$) pH's. The pH's of the syrups from the different modes of crude enzyme applications (HM, HMCB and CES) were not significantly different ($p > 0.05$).

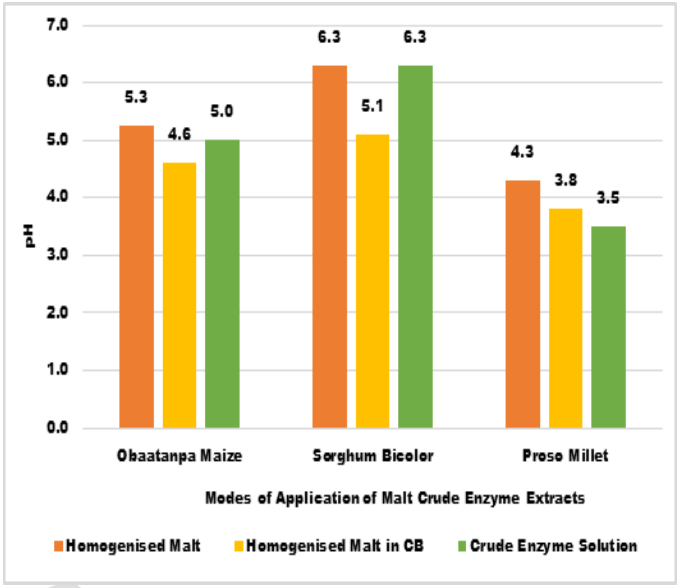

Fig. 9. Three Modes of Applications of Malt Crude Enzyme Extracts from Three Cereals on the pH of Maltose Syrups from Cassava (Esiaba Var.) Starch

D. Percent sulphated ash

The percent sulphated ash contents of the syrups from the different modes of crude enzyme applications (HM, HMCB and CES) were not significantly different ($p > 0.05$).

The percent sulphated ash (Fig. 10) of the maltose syrups from the various malt crude enzymes in this study were: OM (0.09% – 0.11%), SB (0.25% – 0.32%), and MP (0.19% – 0.22%).

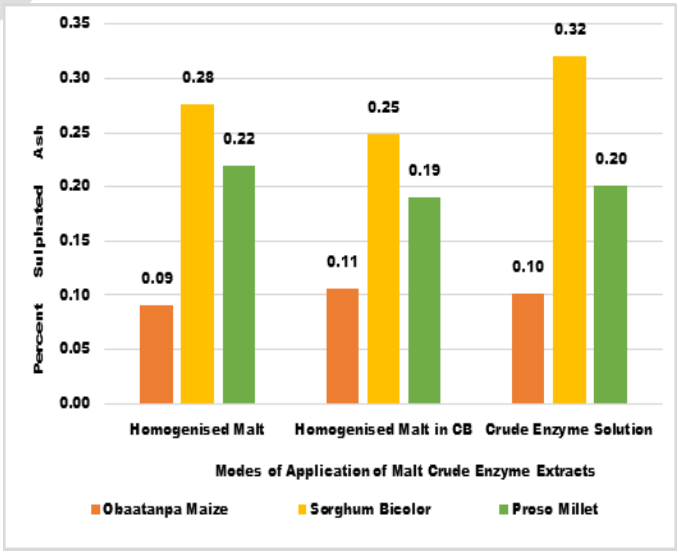

Fig. 10. Three Modes of Applications of Malt Crude Enzyme Extracts from Three Cereals on the Percent Sulphated Ash of Maltose Syrups from Cassava (Esiaba Var.) Starch

These values were lower than the values of 0.45% obtained by Ameko et al (2013) and 0.9% by Bello-Perez et al. (2002) for Banana starch syrup.

IV. CONCLUSION

The crude enzymes from the various cereals were statistically different ($p \leq 0.05$) in their yields of Sweet Juices and resulting maltose syrups with the Obaatanpa Maize (OM) crude enzymes producing the highest yields, followed by the Sorghum Bicolor (SB) crude enzymes, and then the Proso Millet (PM) crude enzymes respectively.

The various modes of application of the crude enzymes were statistically different ($p \leq 0.05$) in their yields of Sweet Juices and resulting maltose syrups with the crude enzyme solutions (CES) producing the highest yields, followed by the homogenised malts enclosed in cheesecloth bags (HMCB). The direct addition of homogenised malts (HM) to gelatinised starch solution gave the lowest yields of maltose syrup.

The DE of the maltose syrups depended on the type of cereal used as the source of malt crude enzymes for hydrolysis of the starch, as well as on the mode of application of the crude enzyme extracts on the gelatinised starch solutions.

The percent reducing sugars and pH of the maltose syrups depended on the type of cereal used as the source of malt crude enzymes for hydrolysis of the starch, but was independent of the mode of application of the crude enzyme extracts on the gelatinised starch solutions.

The percent sulphated ash of the maltose syrups was independent of the type of cereal used as the source of malt crude enzymes for hydrolysis of the starch, as well as of the mode of application of the crude enzyme extracts on the gelatinised starch solutions.

In the production of maltose syrups from cassava starch, the yields of Sweet Juices and resulting maltose syrups, and DE's of the syrups were significantly increased by the use of crude enzyme solutions prepared from malted Obaatanpa maize, Sorghum bicolor and Proso millet respectively, instead of using finely homogenised malts of the cereals for the hydrolysis of the starch.

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