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**Production Of Ascorbic Acid By *Blastoschizomyces spp* and  
*Kluyveromyces marxianus* From Corn Straw Waste Using  
Submerged Fermentation**

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**Abstract**

Ascorbic acid is vital for growth and maintenance of all tissue types in human. This study evaluated the use of Corn Straw Waste (CSW) as a novel substrate for the production of ascorbic acid by yeasts using submerged fermentation. Cells of *Blastoschizomyces spp* and *Kluyveromyces marxianus* were cultured in a liquid fermentation medium containing CSW (0.6 % w/v) for ascorbic acid production. Optimization studies of the ascorbic acid fermentation process was carried out at pH range 4 - 8, temperature range 30 - 45 °C, agitation speed range 50 - 200 rpm for 120 h. Ascorbic acid produced was quantified by titration using 2, 6-Dichlorophenol Indophenol (DCPIP) dye. The statistical analysis of the effects of different concentrations of carbon and nitrogen sources, pH, temperature and agitation speed on ascorbic acid production showed no significant difference at p<0.05. Optimum ascorbic acid yields of 6.5 g/L and 5.5 g/L were produced by *Blastoschizomyces spp* and *Kluyveromyces marxianus* respectively at pH 5, temperature (35 °C) and agitation speed of 100 rpm at 120 h of fermentation. This study shows the potential of Corn straw waste as a novel and economical substrate for ascorbic acid production

**Keywords:** Ascorbic acid; 2, 6-Dichlorophenol Indophenol dye; corn straw; *Blastoschizomyces species*; *Kluyveromyces marxianus*.

**1. Introduction**

Ascorbic acid is an important component of the diet which is needed to prevent scurvy. This organic acid which is white to light- yellow crystal or powder is not only important as an anti- scurvy agent but also acts as antioxidant in bread dough, colour fixing agent, flavouring and preservatives in foods (Higdon, 2006). Ascorbic acid which is required for essential metabolic reactions in living things is made internally by almost all organisms except human beings (Food Standards Agency, 2007). However, several methodologies have been employed in ascorbic acid production. One possible approach to ascorbic acid production is its production from microorganisms. Ascorbic acid production has been reported in *Candida* and *Saccharomyces* species (Huh *et al.*, 1998), *Cryptococcus dimennae* (Kumar, 2000) and *Aspergillus spp* (Banjo *et al.*, 2016). The production of commercially important metabolites by microorganisms using submerged fermentation (SmF) has long been established.

Submerged Fermentation (SmF) otherwise known as Liquid Fermentation (LF) utilizes free flowing liquid substrates,

such as molasses and broths. A significant number of highly economical SmF processes are known to exist not only for enzyme production but also for the production of several other metabolites and are being successfully exploited by industry in most countries of the world. Agricultural wastes can be used as substrate for the production of enzymes and bioactive substances in submerged fermentation.

Corn straw is a very common agricultural waste which consists of the leaves and stalks of maize (*Zea mays*) plants left in a field after harvest. In recent years, one of the most important biotechnological applications is the conversion of agricultural wastes and all lignocellulosic into products of commercial interest such as ethanol, glucose and single cell protein (Ojumu *et al.*, 2003). These wastes can be used as substrate for enzyme production; and also help in solving pollution problems which may be caused by their disposal ( Azeredo *et al.*, 2007; Arvanitoyannis and Varzakas, 2008).

Agricultural waste has been a source of pollution to the environment. Utilization and recycling of these wastes that pose threat to the environment can be systematically converted to useful products. Hence this study exploited the use of corn straw waste as substrate by *Blastoschizomyces spp* and *Kluyveromyces marxianus* in the production of ascorbic acid which is of importance in the pharmaceutical industry.

## 2. Methodology

### Source of Microorganisms

Ascorbic acid producing strains of *Blastoschizomyces spp* and *Kluyveromyces marxianus* were obtained from the Microbiology Laboratory of the Federal University of Agriculture Abeokuta (FUNAAB), Nigeria and sub cultured using Sabouraud Dextrose Agar (SDA).

### Pretreatment of Corn Straw Waste

**Drying of corn straw:** The corn straw obtained from a local farmer at Osiele, Ogun state was dried using hot air oven to approximately 4 % moisture at 80 °C.

**Milling of corn straw:** The dried corn straw was crushed and milled using a waring blender. This was sieved using 40 mm particle size and was later kept in a tight container for further use.

### Production and quantification of ascorbic acid by *Blastoschizomyces spp* and *Kluyveromyces marxianus*

Cells of *Blastoschizomyces spp* and *Kluyveromyces marxianus* were cultured on the corn straw medium (0.8 % Corn

straw, 1 % D - Glucose, 0.15 % L - Galactose, 0.15 % yeast extract, 0.25 % peptone and 0.1 % monosodium glutamate). Ascorbic acid production was monitored at 24 hr. interval for 7 days. Quantitative assay of Ascorbic acid was carried out using the method of Association of Vitamin Chemists (2010).

### **Optimization Of Ascorbic Acid Production by *Blastoschizomyces spp* and *Kluyveromyces marxianus***

**Effect of carbon source (corn straw) on ascorbic acid production:** The effect of Corn straw concentration on ascorbic acid production was studied by incubating *Blastoschizomyces spp* and *Kluyveromyces marxianus* at 30 °C in the fermentation medium containing 1 % D - Glucose, 0.15 % L - Galactose, 0.15 % yeast extract, 0.25 % peptone and 0.1 % monosodium glutamate and varied Corn straw concentration of 0.2 – 1 % (0.2, 0.4, 0.6, 0.8 and 1 %). The ascorbic acid produced was quantified after 120 h of fermentation.

**Effect of nitrogen source (peptone) on ascorbic acid production:** The effect of Nitrogen source on ascorbic acid produced by *Blastoschizomyces spp* and *Kluyveromyces marxianus* was studied at varying concentrations of peptone (0.1, 0.2, 0.3 and 0.4 %). The ascorbic acid produced was quantified after 120 h of fermentation.

**Effect of pH on ascorbic acid production:** Effect of pH on ascorbic acid produced by *Blastoschizomyces spp* and *Kluyveromyces marxianus* was studied at pH range 4.0 – 8.0 (pH 4.0, 5.0, 6.0, 7.0 and 8.0). The ascorbic acid produced was quantified after 120 h of fermentation.

**Effect of temperature on ascorbic acid production:** Effect of temperature on ascorbic acid produced by *Blastoschizomyces spp* and *Kluyveromyces marxianus* was studied at temperature range 30 – 45 °C (30, 35, 40 and 45 °C). The ascorbic acid produced was quantified after 120 h of fermentation.

**Effect of agitation speed:** Effect of agitation speed on the quantity of ascorbic acid formed was studied at different agitation speeds (50,100, 150 and 200 revolution per minute). The ascorbic acid produced was quantified after 120 h of fermentation.

### **Data analysis**

Mean and standard deviation of the duplicated data were analyzed while the significance of the effects of optimization parameters such as different concentrations of carbon and nitrogen sources, pH, temperature and agitation speed were determined using ANOVA at 95% confidence interval while p value <0.05.

## **3. Results and Discussion**

### **3.1 Production of ascorbic acid by *Blastoschizomyces spp* and *Kluyveromyces marxianus***

Studies on the fermentation of the brewery spent grain medium with *Blastoschizomyces spp* and *Kluyveromyces*

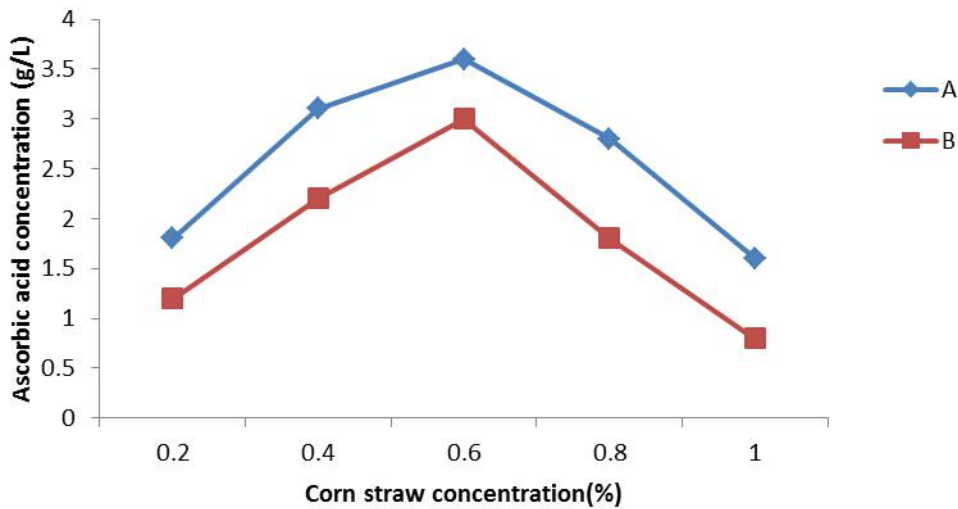
*marxianus* showed that ascorbic acid yield peaked at 120 h of fermentation. Ascorbic acid yields of 3.6 g/L and 3.0 g/L were produced by *Blastoschizomyces spp* and *Kluyveromyces marxianus* at 120 h of fermentation. Thus, 120 h was adopted as the optimum fermentation time for further studies. The yield of ascorbic acid reduced with increase in fermentation time for the two isolates (Table 1). However, at 120 h the yield of ascorbic acid by *K. marxianus* was 0 g/L. This shows that ascorbic acid has been completely degraded in the fermentation medium. The loss of ascorbic acid with fermentation time may be as a result of the increase in the activity of the enzyme ascorbate oxidase that might have been produced by the fermentation microorganism which strongly depends on the pH of the fermentation environment. The enzymes convert ascorbic acid to dehydroascorbic acid (Shindia *et al.*, 2006).

**Table 1: Production of ascorbic acid (g/L) from corn straw waste by *Blastoschizomyces spp* and *Kluyveromyces marxianus***

Isolates	Fermentation time (Hours)										
	12	24	36	48	60	72	84	96	120	132	144
<i>Blastoschizomyces spp</i>	0	0	1.2	2.0	2.2	2.5	2.8	3.2	3.6	2.8	1.2
<i>Kluyveromyces marxianus</i>	0	0.2	0.8	1.4	1.8	2.1	2.2	2.8	3.0	2.2	0

### 3.2 Effect of carbon source (corn straw) on ascorbic acid production

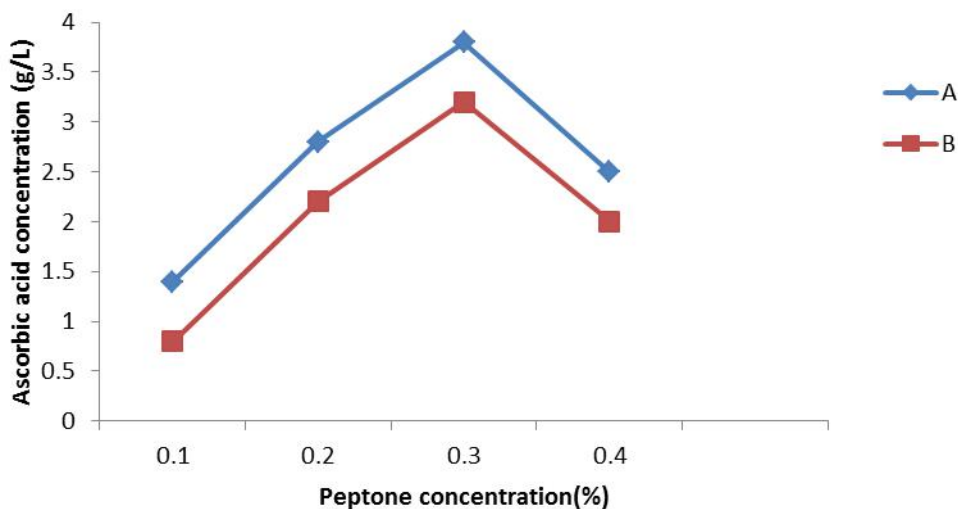
Ascorbic acid accumulation is strongly influenced by the type and concentration of carbon source. The effect of corn straw as carbon source on ascorbic acid production was investigated. Optimum ascorbic acid yield of 3.6 g/L and 3.0 g/L was produced by *Blastoschizomyces spp* and *K. marxianus* respectively at 0.6 % corn straw concentration. There was no significant difference in the ascorbic acid produced at different concentrations of the corn straw waste ( $p < 0.05$ ). However, ascorbic acid production reduced as the concentration of the corn straw was increased (Fig. 1). This result correlates with the findings of Wang *et al.* (2002) who reported that increasing the concentration of carbon source slowed the growth of yeast cells, thus resulting in a reduced secondary metabolite production. According to Leangon *et al.* (2000), carbon source supplementation may be the reason for the over-production of organic acids. In other words glucose is the crucial factor affecting ascorbic acid production.



**Fig. 1: Effect of Carbon source (corn straw concentration) on ascorbic acid production by *Blastoschizomyces spp* (A) and *Kluyveromyces marxianus* (B) (F=0.513, P=0.708)**

**3.3 Effect of nitrogen source (peptone) on ascorbic acid production**

The choice of nitrogen source is of crucial importance for ascorbic acid production. The effect of peptone as nitrogen source on ascorbic acid production revealed that Optimum ascorbic acid production was obtained at 0.3 % of peptone. Optimum ascorbic acid yield of 3.6 g/L and 3.0 g/L was produced by *Blastoschizomyces spp* and *K. marxianus* respectively (Fig. 2). However, there was no significant difference in the ascorbic acid yields at different concentrations of peptone ( $p < 0.05$ ). Ascorbic acid production reduced with increase in the peptone concentration to the fermentation medium.

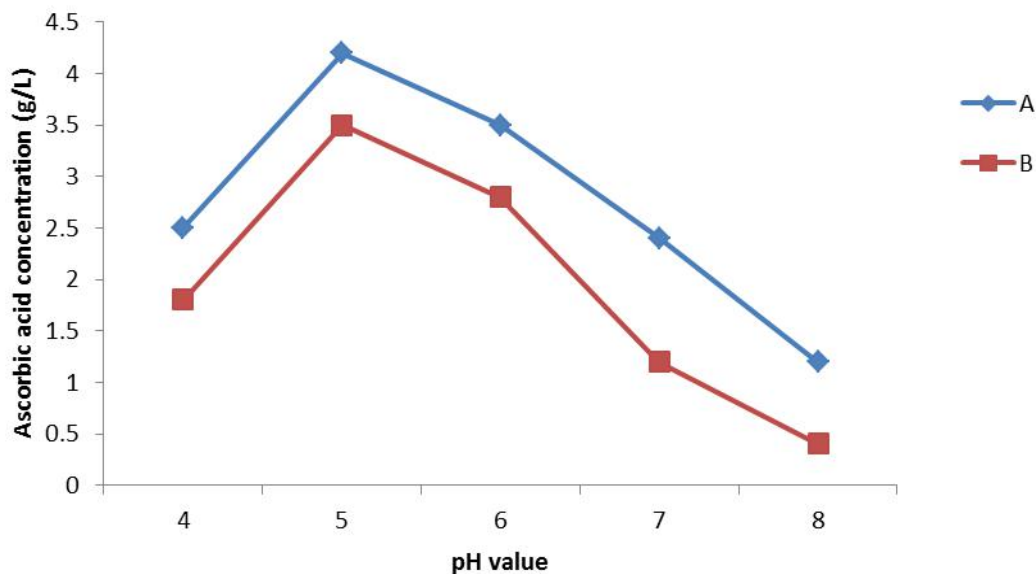


**Fig. 2: Effect of Nitrogen source (peptone concentration) on ascorbic acid production by *Blastoschizomyces spp* (A) and *Kluyveromyces marxianus* (B) (F=0.851, P=0.422)**

**3.4 Effect of pH on ascorbic acid production**

Investigations on the effect of pH on ascorbic acid production showed that there was no significant difference

in ascorbic acid production at pH 5.0 for both isolates ( $p < 0.05$ ). Optimum ascorbic acid yields of 4.2 g/L and 3.5 g/L were produced by *Blastoschizomyces spp* and *K. marxianus* respectively at pH 5.0 (Fig. 3). However, ascorbic acid yield reduced drastically to 1.2 g/L and 0.4 g/L by *Blastoschizomyces spp* and *K. marxianus* respectively as the pH of the medium was increased to pH 8, indicating a decrease in ascorbic acid production beyond the optimum pH of 5. This correlates with a similar work carried out by Shindia *et al.* (2006) who reported the suitability of pH range of 5-6 for organic acid production. Hence, the pH of the culture medium directly influences the growth of microorganisms and the biochemical processes they perform (Sindhu *et al.*, 2009). The findings of the present study is also in line with that of Banjo *et al.* (2008) who reported an optimum pH of 5.0 in their work on ascorbic acid production by *Aspergillus flavus* and *A. tamaritii*. Therefore an environment, which is too acidic, neutral or alkaline, is not conducive for ascorbic acid production.

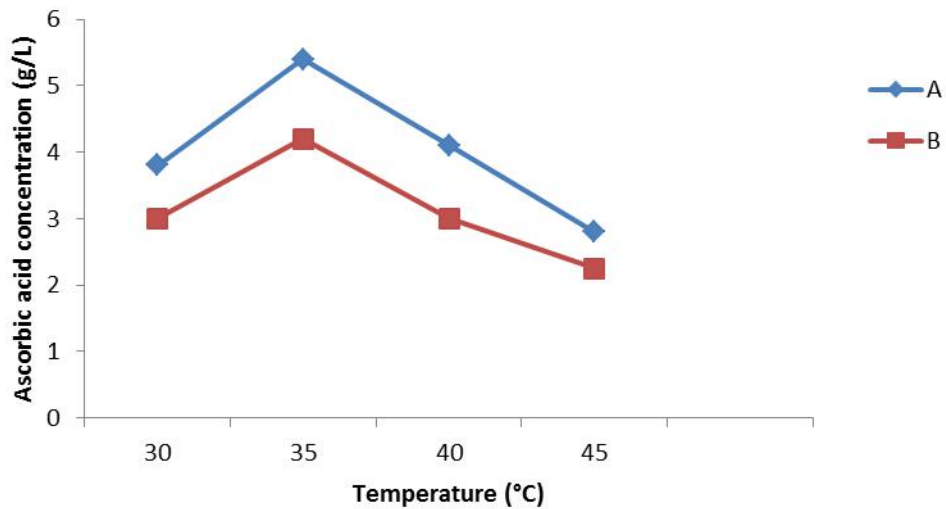


**Fig. 3: Effect of pH on ascorbic acid production by *Blastoschizomyces spp* (A) and *Kluyveromyces marxianus* (B) (F=2.061, P=0.250)**

### 3.5 Effect of temperature on ascorbic acid production

The effect of temperature on ascorbic acid production showed that optimum ascorbic acid yields of 5.4 g/L and 4.2 g/L were produced by *Blastoschizomyces spp* and *K. marxianus* respectively at 35 °C. However, there was no significant difference in the ascorbic acid yields at different temperatures ( $p < 0.05$ ). There was a decrease in ascorbic acid production at higher temperature as shown in Fig. 4. In a related study with another organic acid, Kareem and Rahman (2013) reported reduced citric acid production at temperatures above the optimum temperature. This might be due to accumulation of by-products and eventually, loss of activity as the temperature increases.

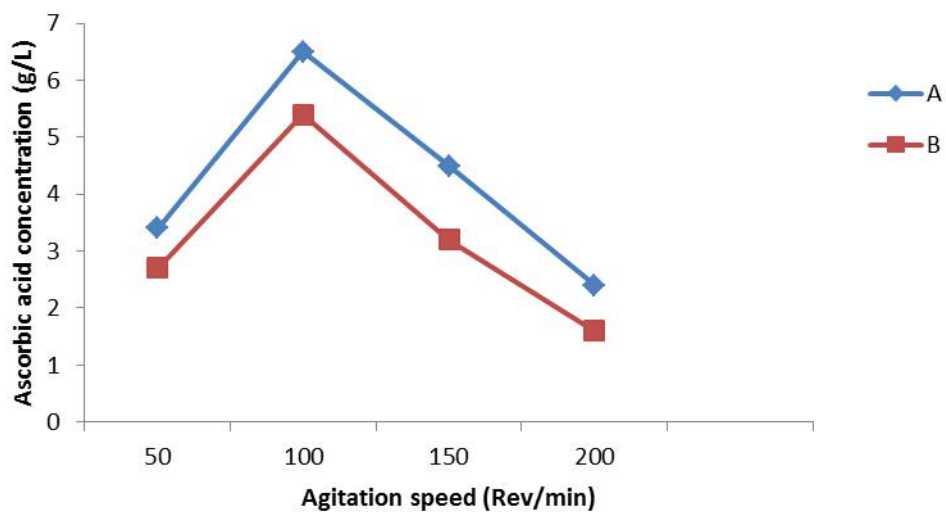
It has been suggested that higher temperatures may cause enzyme deactivation leading to lower ascorbic acid production. Moreover, higher temperature alters the cell membrane composition and stimulates protein catabolism, thus causing cell death. This is in consonance with the findings of Njoku *et al.* (2011) who opined that higher temperature does not favor ascorbic acid production.



**Fig. 4:** Effect of temperature on ascorbic acid production by *Blastoschizomyces spp* (A) and *Kluyveromyces marxianus* (B) (F=0.923, P=0.442)

**3.6 Effect of agitation speed on ascorbic acid production**

A proper agitation speed is important for appropriate air supply and proper mixing of media components, hence the effect of different agitation speed on ascorbic acid production was studied. The study as shown in figure 5 revealed that optimum ascorbic acid yields of 6.5 g/L and 5.5 g/L were produced at an agitation speed of 100 revolution per minute by *Blastoschizomyces spp* and *K. marxianus*. There was no significant difference in the ascorbic acid produced at different agitation speeds ( $p < 0.05$ ). Further increase in agitation speed resulted in reduction in ascorbic acid yield to 2.4 g/L and 1.6 g/L by *Blastoschizomyces spp* and *K. marxianus* at 100 revolution per minute. Increased agitation can impose shear stress on cell walls as well as the cell-insoluble substrate interface. Therefore, an optimum agitation speed is required to maximize product production (Crolla and Kennedy, 2004). At lower agitation speeds, less amount of ascorbic acid produced might be due to improper mixing of the medium (Pena *et al.*, 2008). Different agitation speeds seemed to provide different distribution and transportation of air and nutrients to the cells (Shyam *et al.*, 2009).



**Fig. 5: Effect of agitation speed on ascorbic acid production by *Blastoschizomyces spp* (A) and *Kluyveromyces marxianus* (B) (F=0.415, P=0.590)**

#### 4. Conclusion

In conclusion, this study shows the potentials of yeast cells in the bioconversion of waste to ascorbic acid, which is of great importance to the pharmaceutical industry. The present study showed that optimum ascorbic acid yield of 6.5 g/L and 5.5 g/L were produced by *Blastoschizomyces spp* and *K. marxianus* respectively when cultured on corn straw waste medium at pH 5, 35°C and agitation speed of 100 rpm at 120 h of fermentation. This study established the production of ascorbic acid from an agricultural waste (CSW), thus turning waste to wealth.

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