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RESEARCH ARTICLE

EVALUATING *LACTOBACILLUS ACIDOPHILUS* AND *SACCHAROMYCES CEREVISIAE* COLONY FORMING UNITS IN *ARTEMISIA ANNUA* AND *VERNONIA AMYGDALINA* FERMENTATION: A STUDY ON THE INTERACTIONS OF GROUP, TIME AND CONCENTRATIONMabitine, D.M^a, Iheukwumere, C.C^a, Gberikon, G.M^b, Ogbonna, I.O^b, Ogbonna, C.I.C^b Ojobo, O.A^a, Edinoh, D.O^{a*}, Ado, B.V^b, Ahembe, N^a, Adejo, H.I^a, Onoja, E.V^a, Ujiji, D^a, Joshua, D^a, Ngwu, J.E^b, Jacob, M^a^aDepartment of Plant Science and Biotechnology, College of Biological Sciences, Joseph Sarwuan Tarka University (JOSTUM), Makurdi, Benue State^bDepartment of Microbiology, College of Biological Sciences, Joseph Sarwuan Tarka University (JOSTUM), Makurdi, Benue State.*Corresponding Author Email: edinohdaniel1@gmail.com

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ARTICLE DETAILS

ABSTRACT

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Microbial quality and quantity in fermentation is critical to obtaining value added products. This research aimed at investigating the interactive effect of group, day and concentration in the fermentation of leaf extracts of *A. annua* and *V. amygdalina* into more value added products. The aqueous leaf extracts of *A. annua* (A.AAQ) and aqueous *V. Amygdalina* (V.AAQ) were fermented with *Saccharomyces cerevisiae* and *Lactobacillus acidophilus* respectively for 35 days and evaluated for Colony Forming Units (CFU). Data were statistically analyzed using analysis of variance to compare and separate the means. The effect and interactive effect of group alone, group and day, group and concentration, group, day and concentration, group and concentration on CFU of *Saccharomyces cerevisiae* and *Lactobacillus acidophilus* were not significantly different ($p > 0.05$). On the other hand, the effect of day, concentration, day and concentration on CFU of *Saccharomyces cerevisiae* as well as effect of group on *Saccharomyces cerevisiae* and *Lactobacillus acidophilus* were significantly different ($p < 0.05$; 0.001). The effect of day, concentration and interactive effect of day and concentration on CFU of *Lactobacillus acidophilus* were significantly different ($p < 0.05$; 0.001). In conclusion, the effect of day and concentration as well as the interactive effect of day and concentration may have significantly affected the CFU of both organisms used in the fermentation there by accounting for facilitating the fermentation process.

KEYWORDS

Fermentation, Microbial Quality and Quantity, Colony Forming Units (CFU), *Artemisia annua*, *Vernonia amygdalina*, *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, Interactive Effects (Day and Concentration).

1. INTRODUCTION

Fermentation is affected by types of microorganisms involved and their Colony Forming Units. Other factors are the concentration and composition of the plants extracts, pH, temperature among others. Colony Forming Units (CFU) allows producers, manufacturers and researchers to monitor and control fermentation. The higher the CFU, the higher the fermentation rate and the better the flavour, taste, and other desired qualities (Sharma et al., 2020). These microbial actions assist in the conversion of multifarious sugar to alcohols, proteins to peptides and amino acids as well as oils and fats to fatty acids and glycerol. Consequently, it enhances the provision of food security, good health, higher income source and general industrial growth (Ross et al., 2018). Factors which ease fermentation are type of microorganisms used, type and composition of plant extracts used, fermentation time and experimental conditions and equipment used (Nkhata et al., 2018; Ross et al., 2018). The process often decreases cooking time of fermented and supports positive reforms leading to value-added feel, eminence, flavour, colour, aroma, longer shelf life and nourishing value (Nkhata et al., 2018; Ross et al., 2018). Some of the microorganisms used in fermentation are *Lactobacillus*, yeasts, *Debaryomyces*, *Saccharomyces*, *Mucor* and *Rhizopus* species. These and other microorganisms exhibit activities which enhance digestibility and availability of nutrients during fermentation due

to the enzyme activity from the microbe (Nkhata et al., 2018; Xiang et al., 2019; Fernandes et al., 2020). documented that the bacteria count varied with the total count ranged from 3.265 to 7.254 log CFU/mL. The study opined that in the evaluation of microbial diversity of fermented Tapai (an alcoholic drink), there was an increase in CFU/g of 10^5 and 10^6 in yeast and Lactic Acid Bacteria after two weeks of fermentation (Adacsi et al., 2022; Chiang et al., 2006). In this work, the extracts of *A. annua* var. *chiknensis* with batch number (PCN 004651/PAC4.8/CIC) and *V. amygdalina* reported to have antidiabetic and other properties will be fermented with *S. cerevisiae* and *Lactobacillus acidophilus* (Ogbonna et al., 2017).

2. MATERIAL AND METHODS

2.1 Study Area

This study was performed in the College of Biological Sciences Joseph Sarwuan Tarka University, Makurdi and Evidence Laboratory Makurdi, Benue State.

2.2 Collection and identification of plant materials

The leaves of *A. annua* and *V. amygdalina* were obtained from Centre for Biotechnology and Genetic Engineering (CBGE) University of Jos, Jos

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Plateau State and the farm behind Modern Market Makurdi, Benue State respectively.

The *A. annua* var. *chiknensis* with batch number (PCN 004651/PAC4.8/CIC). The *V. amygdalina* was identified in the taxonomy unit in the Department of Botany, Joseph SarwuanTarka University, Makurdi. They were rinsed with clean water, shade dried at room temperature and stored in glass bottles until needed for use.

2.3 Preparation of extracts

Leaf powder of *A. annua* and *V. amygdalina* (400g each) was poured into conical flask enclosing 2000ml of sterilized condensed water and heated with a Bunsen flame for 10 minutes and cooled to room temperature. All the set up were aseptically filtered using Whatman filter paper (No1) to separate the residue from the filtrate and allowed to dry on hot water bath. The extracts were deposited in a refrigerator at 5 °C.

2.4 Preparation of fermented plant samples

Previously boiled water was dispensed into a sterile flask, left for 2-3 minutes and again poured into sterile bottles. Leaf extracts (20g each of *A. annua* and *V. amygdalina*) were discharged into bottles containing 300ml each of sterile distilled water. Ten (10) ml each of *S. cerevisiae* and *Lactobacillus acidophilus* culture were added to different bottles containing the extract of *A. annua* and *V. amygdalina* respectively, airtight and preserved in a dark environment unperturbed for fourteen days.

Thereafter, racking was performed by decanting the clear part into another sterile vessel and racked again after another fourteen days and then after seven days. After thirty five days, the contents in the bottle were left to age.

2.5 Total heterotrophic count analysis of the extracts of A. annua and V. amygdalina

Peptone (1g) was poured into a sterile glass container followed by addition of 1000mL of distilled water and was robustly stirred to form a solution. Then 0.5-1.0% of NaCl was transferred into the solution for extra osmotic equilibrium and finally sterilized by autoclaving at 121°C for 15 minutes and 15psi pressure. A serial dilution of between 10⁻¹ to 10⁻⁵ was then made using 1ml of each fermented sample.

The different dilutions of the fermented *A. annua* and *V. amygdalina* were plated on already prepared Sabouraud Dextrose Agar (SDA) and De Man Rogosa Sharpe Agar (MRSA) agar respectively and allowed to incubate for between 24 to 48 hours in an anaerobic and aerobic condition at 37°C (Cheesebrough, 2006). To the Sabouraud Dextrose Agar (SDA), 0.05mg/ml of streptomycin was poured to stop bacterial growth.

The extracts were allowed to ferment for thirty five days each. The colonies obtained were sub-cultured on new culture agar to get pure cultures of *S. cerevisiae* and *L. acidophilus* by incubating at 37°C and 25°C respectively for between 24 to 48 hours.

The colonies were counted manually and the CFU/mL were evaluated using the formula

$$CFU/mL = \frac{\text{Number of colonies} \times \text{Dilution factor } 2x \dots x \text{Dilution factor } n}{\text{Volume of sample plated mL}}$$

2.6 Statistical Analysis Of Results

Data gotten were analysed with R-Studio software and expressed as mean ± Standard Error of Mean (SEM). The Analysis of variance (ANOVA) was used to compare the means. Statistical significance was established at p<0.05 and LSD was used to separate the mean.

3. RESULTS

There was no significant effect of the group (extracts) on the Colony Forming Unit (CFU) of *S. cerevisiae* and *L. acidophilus* (p>0.05) (Table 1).

The effect of days on the CFU of *S. cerevisiae* during fermentation was

significantly different (p<0.001) (Table 2).

The effect of concentration on the CFU of *Lactobacillus acidophilus* during fermentation was significantly different (p<0.001) (Table 3).

The interactive effect of group and day on the CFU of *S. cerevisiae* and *L. acidophilus* was not significantly different (p>0.05) (Table 4).

The interactive effect of group and concentration on the CFU of *S. cerevisiae* and *L. acidophilus* was not significantly different (p>0.05) (Table 5).

The interactive effect of day and concentration on the CFU of *S. cerevisiae* significantly different (p>0.001) (Table 6).

The effect of group on the CFU of *S. cerevisiae* and *L. acidophilus* isolates was significantly different (p<0.05) (Table 7).

The effect of day on the CFU of *Lactobacillus acidophilus* not significantly different (p>0.05) (Table 8).

The effect of concentration on the CFU of *Lactobacillus acidophilus* significantly different (p<0.001) (Table 9).

The interactive effect of group and day on the CFU of *Lactobacillus acidophilus* and *S. cerevisiae* not significantly different (p>0.05) (Table 10).

The interactive effect of day and concentration on the CFU of *Lactobacillus acidophilus* was significantly different (p<0.001) (Table 11).

The interactive effect of group and day on the CFU of *Lactobacillus acidophilus* and *S. cerevisiae* was not significantly different (p>0.05) (Table 12).

Table 1: Effect of Group on Colony Forming Units (CFU/mL) of Lactobacillus acidophilus and S. cerevisiae

Group	CFU	p-value
A.AF	28.08 ± 3.31	> 0.05
V.AF	25.17 ± 3.35	

Values are means and standard error of means (Mean ± SEM) of three replicates. Mean values not significantly different (p>0.05)

A.AF= *A. annua* fermented V.AF= *V. amygdalina* fermented

Table 2: Effect of Day on Colony Forming Units (CFU/mL) of S. cerevisiae

Day	CFU	p-value	LSD
1	15.67 ± 2.24 ^c	< 0.001	4.67
14	26.06 ± 3.6 ^b		
28	38.22 ± 5.36 ^a		
35	26.56 ± 5.5 ^b		

Values are means and standard error of mean (Mean ± SEM) of three replicates. Mean values with superscript down the column are significantly different (p<0.001).

Table 3: Effect of Concentration on Colony Forming Units (CFU/mL) of S. cerevisiae

Concentration	CFU	p-value	LSD
1/100	47.83 ± 3.77 ^a	< 0.001	4.04
1/1000	22.63 ± 1.47 ^b		
1/10000	9.42 ± 1.2 ^c		

Values are means and standard error of mean (Mean ± SEM) of three replicates. Mean values with superscript down the column are significantly different (p<0.001).

Table 4: Interactive Effect of Group and Day on Colony Forming Units (CFU/mL) of L. acidophilus and S. cerevisiae

Group	Day	CFU	p-value
A.AF	1	16.33 ± 3.06	> 0.05
A.AF	14	26.44 ± 5.21	
A.AF	28	39.78 ± 8.08	
A.AF	35	29.78 ± 7.42	

Table 4 (Cont): Interactive Effect of Group and Day on Colony Forming Units (CFU/mL) of <i>L. acidophilus</i> and <i>S. cerevisiae</i>		
V.AF	1	15 ± 3.44
V.AF	14	25.67 ± 5.27
V.AF	28	36.67 ± 7.51
V.AF	35	23.33 ± 8.41

Values are means and standard error of mean (Mean ± SEM) of three replicates. Mean values down the column are not significantly different (p>0.05)

Table 5: Interactive Effect of Group and Concentration on Colony Forming Unit (CFU/mL) of <i>L. acidophilus</i> and <i>S. cerevisiae</i> .			
Group	Concentration	CFU	p-value
A.AF	1/100	49 ± 5.62	> 0.05
A.AF	1/1000	24.17 ± 1.96	
A.AF	1/10000	11.08 ± 1.34	
V.AF	1/100	46.67 ± 5.25	
V.AF	1/1000	21.08 ± 2.19	
V.AF	1/10000	7.75 ± 1.94	

Values are means and standard error of mean (Mean ± SEM) of three replicates. Mean values down the column are not significantly different (p>0.05)

Table 6: Interactive Effect of Day and Concentration on Colony Forming Unit (CFU/mL) of <i>S. cerevisiae</i>				
Day	Concentration	CFU	p-value	LSD
1	1/100	24 ± 0.78 ^{de}	< 0.001	8.086
1	1/1000	19.17 ± 2.21 ^{ef}		
1	1/10000	3.83 ± 1.11 ^s		
14	1/100	45.17 ± 2.75 ^c		
14	1/1000	21.83 ± 1.62 ^e		
14	1/10000	11.17 ± 0.87 ^{fg}		
28	1/100	66.17 ± 5.66 ^a		
28	1/1000	31.83 ± 1.82 ^d		
28	1/10000	16.67 ± 0.88 ^{ef}		
35	1/100	56 ± 5.07 ^b		
35	1/1000	17.67 ± 2.16 ^{ef}		
35	1/10000	6 ± 2.05 ^s		

Values are means and standard error of mean (Mean ± SEM) of three replicates. Mean values with superscript down the column are significantly different (p<0.001).

Table 7: Effect of Group on Colony Forming Units (CFU/mL) of <i>L. acidophilus</i> and <i>S. cerevisiae</i>			
Group	CFU	p-value	LSD
A.AF	28.08 ± 3.31 ^a	< 0.05	2.08
V.AF	25.17 ± 3.35 ^b		

Values are means and standard error of mean (Mean ± SEM) of three replicates. Mean values with superscript down the column are significantly different (p<0.05).

Table 9: Effect of Concentration on Colony Forming Units (CFU/mL) of <i>Lactobacillus acidophilus</i>			
Concentration	CFU	p-value	LSD
1/100	46.75 ± 2.59 ^a	< 0.001	2.54
1/1000	21.48 ± 0.98 ^b		
1/10000	9.08 ± 0.83 ^c		

Values are means and standard error of mean (Mean ± SEM) of three replicates. Mean values with superscript down the column are significantly different (p<0.001).

Table 8: Effect of Day on Colony Forming Units (CFU/mL) of <i>L. acidophilus</i>			
Day	CFU	p-value	LSD
1	14.78 ± 1.53 ^c	< 0.001	2.94
2	25.56 ± 2.53 ^b		
3	37 ± 3.63 ^a		
4	25.75 ± 3.76 ^b		

Values are means and standard error of mean (Mean ± SEM) of three replicates. Mean values with superscript down the column are significantly different (p<0.001).

Table 10: Interactive Effect of Group and Day on Colony Forming Units (CFU/mL) of <i>Lactobacillus acidophilus</i>			
Group	Day	CFU	p-value
A.AF	1	15.22 ± 2.08	> 0.05
A.AF	2	25.56 ± 3.56	
A.AF	3	38.11 ± 5.35	
A.AF	4	28.78 ± 4.99	
V.AF	1	14.33 ± 2.3	
V.AF	2	25.56 ± 3.71	

Table 10: Interactive Effect of Group and Day on Colony Forming Units (CFU/mL) of <i>Lactobacillus acidophilus</i>		
V.AF	3	35.89 ± 5.04
V.AF	4	22.72 ± 5.67

Values are means and standard error of mean (Mean ± SEM) of three replicates. Mean values without superscript down the column are not significantly different (p>0.05)

Table 11: Interactive Effect of Group and Concentration on Colony Forming Units (CFU/mL) of <i>Lactobacillus acidophilus</i> and <i>S. cerevisiae</i>			
Group	Concentration	CFU	p-value
A.AF	1/100	47.54 ± 3.78	> 0.05
A.AF	1/1000	22.63 ± 1.32	
A.AF	1/10000	10.58 ± 0.95	
V.AF	1/100	45.96 ± 3.63	
V.AF	1/1000	20.33 ± 1.45	
V.AF	1/10000	7.58 ± 1.32	

Values are means and standard error of mean (Mean ± SEM) of three replicates. Mean values down the column are not significantly different (p>0.05)

Table 12: Interactive Effect of Day and Concentration on Colony Forming Units (CFU/mL) of <i>Lactobacillus acidophilus</i>				
Day	Concentration	CFU	LSD	p-value
1	1/100	23.25 ± 0.61 ^e	5.0860	< 0.001
1	1/1000	17.83 ± 1.42 ^f		
1	1/10000	3.25 ± 0.68 ^h		
14	1/100	45 ± 1.84 ^c		
14	1/1000	21.08 ± 1.02 ^{ef}		
14	1/10000	10.58 ± 0.53 ^g		
28	1/100	64.25 ± 3.8 ^a		
28	1/1000	30.25 ± 1.08 ^d		
28	1/10000	16.5 ± 0.49 ^f		
35	1/100	54.5 ± 3.6 ^b		
35	1/1000	16.75 ± 1.44 ^f		
35	1/10000	6 ± 1.3 ^{gh}		

Values are means and standard error of mean (Maen ± SEM) of three replicates. Mean values with superscript down the column are significantly different (p<0.001).



Plate 2: Culture plate of *L. acidophilus*



Plate 2: Culture plate of *L. acidophilus*

4. DISCUSSIONS

Group, day and concentration showed various effects on the CFU of the isolates of *S. cerevisiae* and *L. acidophilus* during fermentation. Day (fermentation period) and concentration of extracts both individually and interactively significantly increased the CFU of *S. cerevisiae* in the first four weeks of fermentation. This may have facilitated fermentation rates which resulted in the synthesis of some bioactive compounds and introduction of flavors and other desirable qualities. However, the cells of *S. cerevisiae* decreased in the fifth week of fermentation possibly due to increase in acidity as a result of a decrease in pH. Also, the group (extract) and concentration of the extract individually significantly increased the CFU of *L. acidophilus*. The interactive effect of fermentation period (days) and concentration significantly increased the cells of *L. acidophilus*. This is supported by the report who documented similar findings of (Bello and Ojokoh, 2013).

Analysis has published several features of *Lactobacillus acidophilus* and *S. cerevisiae*. These physiognomies may have facilitated the survival of these organisms in extreme environmental conditions thereby supporting their growth which in may have enhanced the fermentation. These probiotics have industrial and other important functions (Park et al., 2007; Kechangia et al., 2013 and Delgado-Ospino et al., 2022; Rahmati, 2017).

A decrease in pH may have aided the rise in CFU of the starter organisms leading to increasing fermentation rate. While decrease in pH adversely influences the microbes in a wide variety of ways (Charkraborty and Kenney, 2018), these microbes often adopt coping strategies such as use of the enzyme-catalyzed reactions in order to destroy proton from the cellat the cost of ATP intake, manufacture of complexes to neutralise low pH (Shabala and Ross 2008; Mira et al., 2010).

5. CONCLUSION

CFU is very important in fermentation. In this study, the effect of day and concentration as well as the interactive effect of day and concentration were found to significantly affect the CFU of both organisms used in the fermentation there by accounting for facilitating the fermentation process resulting in end products which have undergone several value added modifications for domestic and industrial purposes.

Authors contributions

Iheukwumere, C.C, Gberikon, G.M, Ogbonna, I.O, Ogbonna, C.I.C, developed the concepts and managed the research; Mabitine Daniel Malo collected data, peromed the experiments and wrote the first draft of the manuscript; Ojobo, Omoche performed the statistical analysis; Edinoh Daniel, Ado Benjamin and all authors reviewed the draft of the manuscript; all authors approved manuscript accomplishment.

REFERENCES

Adacsi, C., Kovacs, S., Pocsi, I., Gyori, Z., Dombradi, Z., and Pusztahelyi, T., 2022. Microbiological and Toxicological Evaluation of Fermented Forages. *Agricultural*, 12: Pp. 421

Bello, B K., Ojokoh, A O., 2013. Effect of fermentation on the microbial growth and proximate composition of water yam. *International Journal of Biosciences and Technology; Coimbatore* 6(2): Pp. 9-13

- Cheesebrough, M., 2006. District Laboratory Practice in Tropical Countries. Second Edition update part one. Cambridge University Press. Pp. 454
- Chiang Y.W., Chye, F.Y., and Mohd Ismail, A., 2006. Microbial and Proximate Composition of Tapai, a Sabah' fermented Beverage. Malaysian Journal of Microbiology. 2(1): Pp. 1-6
- Dilgado-Ospina, J., Puerta-Polanco, L.F., Grande-Tovar, C.D., Cuervo, R.A and Chaves-Lopez, 2022. Exploring the Core Microbiota of four different traditional fermented Beverages from Colombian Aides. Fermentation 8(12): Pp. 733
- Fernandes, A.M.O., Garcia, N.F.L., Leite, R.S.R., and Fossada Paz, M., 2020. Evaluation of the Fermentative Capacity of *S. cerevisiae* and *P. kudriavzevii* BB2 at Simulated Industrial Conditions. Indian Journal of Microbiology 60(4)
- Kechangia, M., Basoulis, D., Konstanopoulou, S., Dimitriadi, D., Gyftopoulou, K., Skarmoutsou, N., and Fakiri, E.M., 2013. Health benefits of probiotics A review. ISRN Nutrition. Article ID:481651
- Mira, N.P., Teixeira, M.C., and Sa-Correia, I., 2010. Adaptive response and tolerance to weak acids in *S. cerevisiae*; a genome-wide view OMICS 14: Pp., 525-540
- Nkhata, S.G., Ayua, E., Kamau, E.H., Shingiro, J.B., 2018. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Science and Nutrition. 6: Pp. 2446-2458.
- Ogbonna, C.I.C., Ogbonna, A.I., Onyimba, I.A., Itelima, J.V., Umar, A.F., Onyezili, N., Istifanus, F., 2017. Combined Antidiabetic Effects of Extracts of *Artemisia annua* L var. *chiknensi* (BGE/CHNA/09/LTNGS/G) and Each of Three other Plants (*Momordica charantia* Linn. *Vernonia amygdalina* Del. and *Aegle marmelos* Correa) Traditionally used in Nigeria for the Treatment of Diabetes. Journal of Scientific Research and Report 16(2): Pp. 1-12.
- Park, Y.H., Kim, J.G., Shin, Y.W., Kim, S.H., and Whang, K.Y., 2007. Effect of Dietary inclusion of *Lactobacillus acidophilus* ATCC43121 on Cholesterol Metabolism in rats. Journal of Microbiology and Biotechnology 17: Pp. 655-662
- Rahmati, F., 2017. Characterization of *Lactobacillus*, *Bacillus* and *Saccharomyces* isolated from Iranian traditional dairy products for potential sources of starter cultures. AIMS Microbiology. 3(4): Pp. 815-825
- Ross, C., Robert, D.L., Elizabeth, B., 2017. Given Diabetes is fat better than thin, Diabetes Care. 20 (4): Pp. 650-652.
- Shabala, L., and Ross, T., 2008. Cyclopropane fatty acids improve *E. coli* survival in acidified minimal media by reducing membrane permeability to H⁺ and enhanced ability to extrude H⁺. Resource Microbiology 159: Pp. 458-461
- Sharma, J., Garg, P., Kumar, P., Bhatia, S.K., and Kulshrestha, S., 2020. Microbial Fermentation and its role in quality improvement for fermented foods. Fermentation. 6:106; doi:10.3390/fermentation6040106

