Acta Scientific NUTRITIONAL HEALTH (ISSN:2582-1423)

Volume 7 Issue 7 July 2023

Review Article

Lactic Acid Produced by Wheat Straw Fermentation with Rhizopus Arrhizus Extends the Keeping Time of Fresh Poultry Meat

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DOI: 10.31080/ASNH.2023.07.1259

Received: May 09, 2023

Published: June 07, 2023

Abstract

Wheat straw is an abundant renewable lignocellulosic biomass. It can serve as substrate for numerous biotechnological processes. Wheat straw was used as substrate for lactic acid production with *Rhizopus arrhizus* in submerged fermentation. On-site enzymatic Saccharification using *Trichoderma viride* and subsequent sugar fermentation were performed for the production of lactic acid. 100 gL-1 of pretreated wheat straw slurry at 50 ± 1 °C was subjected to enzymatic hydrolysis. The obtained hydrolysate was then subjected to fermentation at 35 ± 1 °C for 7 days at an agitation speed of 180 rpm, pH was adjusted to 5.0 after which 10% inoculum was added. Product was analyzed using HPLC method. 19.25 gL^{-1} of Lactic acid was generated with a % yield of 55.24%. Meat samples were then treated by soaking in varying concentration (1%, 2%, 3% and control) of lactic acid (n = 4). Treated samples were then stored in HDPE films at 4°C for 14 days. TVC, TCC, TVB-N and TBARS were evaluated during storage. pH, meat acid-activity and sensory parameters were also evaluated for each treatment. Significant differences in the TVC and TCC were observed within the treatments ($P < 0.05$) as storage progressed. Treatment with 3% gave the lowest TVC and TCC of 1.98 ± 0.30 and 0.04 ± 0.04 log10 CFU g-1 on day 8 and 7 respectively. 2% lactic acid concentration also gave the highest keeping time of 10 days, while maintaining maximum sensory attributes (above 17.00). Hence, the bio-conversion of lignocellulosic waste with microorganisms can be adopted for the generation of useful industrial products.

Keywords: Lignocellulosic-Wastes; Organic Acids; Fermentation; Meat Preservation; Shelf-Life

Introduction

Lignocellulosic materials such as straws, stalks and shells represents an abundant natural renewable carbon resource for various fermentation processes with long term sustainability [1]. The growing interest in the utilization of lignocellulosic materials from different agricultural, foods, as well as domestic/municipal solid wastes have greatly generated both economic and environmental impact; specifically, this relates to the reduction of land used for disposal as well as *valorization* or value addition of wastes and use as raw materials for various bio-production processes.

Wheat straw which is a by-product of wheat farming, is a typical lignocellulosic biomass that is globally available and can serve as an ideal substrate for the generation of bio-based products, owing to its high cellulose composition (24 - 40% of dry weight), as well as its abundance (778 million metric tons annually produced) [2]. Its exploitation and transformation has been inadequate [3]. Several applications of wheat straw in biotechnological processes have been reported, and this has been achievable on industrial scale by adopting solid-state fermentation (SFF) or submerged fermentation (SMF) since wheat straw contains basic nutrients required for microbial growth [4].

Lactic acid can be described as a value-added product obtainable from renewable sources, it is regarded as the most widely known hydroxy carboxylic acid present in almost all organisms, hence, its production is of utmost necessity. Lactic acid can be grouped into L-, D-, and DL-lactic acids, according to its configuration and optical rotation. It has however been found that only L-lactic acid can be properly metabolized by the human body without the generation of toxic compounds/side effects, due to the presence of L-lactic acid dehydrogenation enzyme. Excessive consumption of D- or DL-lactic acid has been known to cause acute toxicity [5]. There are two methods employed in the production of lactic acid; the first in-

volves the chemical synthesis from fossil fuels, which produces only DL-lactic acid, while the second is biotechnology-based, it involves fermentation of sugars, which is capable of producing a particular lactic acid by selecting specific microorganisms, substrates, and conditions [6]. Agricultural residues are currently utilized for the production of lactic acid and other bio based products to decrease total dependence food crops and forest woody biomass, hence preventing continuous deforestation [7-9].

L-lactic acid is a commercial product with a wide range of application which includes pharmaceutical (dialysis solution, mineral preparations, tableting, surgical sutures, and prosthesis), chemical (pH regulators, chiral intermediates, cleaning agent, and a green solvent), food (acidulant, flavor enhancer, preservatives, mineral fortification, and antioxidant) and cosmetic (anti-tartar agents, anti-acne agents moisturizers, skin-lightening agents, skin rejuvenating agents, and humectants) [10]. Poultry meat, which is believed to be a perishable product is highly susceptible to spoilage in the form of discoloration, off odours/taste and altered viscosity during storage at ambient conditions. Foodborne illnesses resulting from poultry meat contamination has also become a major source of global concern. *Salmonella* and *Campylobacter* causes more foodborne illnesses in poultry than any other bacterial [11]. It was estimated that 1 in every 25 packages of chicken at the grocery store is contaminated with *Salmonella* [12]. Verotoxin producing *Escherichia coli* 0157:H7 (VTEC), *Listeria* and *Yarsinia* have become prominent in some areas as additional foodborne pathogens. A number of other toxigenic pathogens such as *Clostridium perfringes, Bacillus cereus* and *Staphylococcus aureus* can also enter the food chain via contaminated poultry products [11]. Several regulations have been passed by the European Parliament Council Regulation (EPCR) on the control of several foodborne zoonotic agents, which covers the adoption of certain regulations aimed at reducing the prevalence of specified zoonosis in food animals at the level of primary production. These infections are distributed worldwide and result in severe economic losses when no effort is made towards their control. It is therefore paramount to employ proper and adequate methods in preservation to prevent such changes and hence prolong its storage time.

Studies on the application of acids in food preservation have been carried out, researchers have investigated the efficacy of various acids preservation on meat surfaces during storage [13,14]. Studies have also been carried out to determine the possibility of inhibiting microbial proliferation by engaging acids producing bacteria on the surfaces of meat products [15]. Microbial proliferation and chemical spoilage are the two major causes of reduced shelf-life in fresh poultry meat during refrigeration storage, therefore the employment of adequate preservative agents in the treatment of meat surfaces could go a long way in inhibiting microbial growth [16]. Owing to its ability to alter the proton motive force (PMF) generated on the cell surfaces of microorganisms, Lactic acid has a potential to be highly effective in meat preservation if applied optimally in meat treatments [16]. Hence lactic acid produced from lignocellulosic waste using *R. arrhizus* in submerged fermentation can be used as a preservative agent, which will not only help to reduce environmental wastes but will also preserve meat from post-slaughter spoilage. This study is therefore aimed at producing lactic acid using wheat straw as substrate with *Rhizopus arrhizus* in submerged fermentation and to assess its preservative potential on fresh poultry meat samples.

Materials and Method

Materials and reagents

Wheat straw was collected from farms within south western Nigeria. The collected material was cut into pieces, milled, and sieved to obtain 40 to 60 mesh fractions. The samples were then homogenized and stored in plastic bags for further use. Poultry meat samples were obtained from freshly slaughtered chickens at a local poultry farm in Akure, Ondo state, Nigeria. NaOH, HCl, distilled water, plate count agar, violet red bile glucose agar (VRBGA). (All reagents used were of Sigma brand, Darmstard, Germany).

Microorganism

Rhyzopus arrhizus (OQ690654) and *Trichoderma viride* (OQ686701) used in this study were cultured in the Department of Microbiology, Federal university of technology, Akure, Nigeria. The microorganisms were maintained on PDA (Potato-Dextrose agar).

Sample preparation

Poultry meat samples were obtained from freshly slaughtered broiler chickens, the meat samples were aseptically deboned, defatted and cut into strips, using sterilized utensils. Prepared meat strips were then packed into sterile polyethylene bags, sealed and rapidly transferred to the laboratory in ice packs for immediate treatment.

Inoculum preparation

Rhizopus arrhizus was grown in Erlenmeyer flasks with 100 ml of liquid media containing; glucose, 20 gL^{-1}; $(NH_4)_2SO_4$, 2 gL^{-1}; ZnSO4·7H2O, 0.05 gL^{-1}; FeSO4, 0.018 gL^{-1}; KH_2PO_4, 0.3 gL^{-1}; and $MgSO_4$, 0.3 gL^{-1}. The flasks were incubated on an incubator shaker (MRC laboratory instruments, Israel) continuously at 160 rpm and 35 °C for 18 hours before use [17].

Dilute acid pretreatment

Dilute acid pretreatment of wheat straw was carried out using a modified method of Mood., *et al.* [18]. Previously milled samples straw was deacetylated using a dilute NaOH solution (0.4% w/w) at 80 °C for two hours, after which solids were washed with water

and then dilute H_2SO_4 solution was added to achieve a 0.8% (w/w) acid concentration for dilute acid pretreatment. The slurry was vigorously stirred for two hours at room temperature, dewatered to approximately 40% solids and then incubated in a horizontal pretreatment reactor at 140°C with a residence time of 10 min. After pretreatment, the material was then separated into the slurry stream with high solid content and volatile flash vent stream. Pretreated deacetylated dry slurry was then neutralized using a 50% NaOH solution.

Enzyme extraction and assay

The fungi specie *Trichoderma viride*, was used as a source of cellulases. For cellulases production, 150 mL liquid medium containing: $(NH_4)_2SO_4$, 1.4 gL^{-1}; urea, 0.3 gL^{-1}; KH_2PO_4, 2.0 gL^{-1}; $MgSO_4$.7H2O, 0.3 gL^{-1}; $CaCl_2$, 0.3g; Tween 80, (0.2%); wheat straw powder, 20g; cellulose powder, 8g; and 1 ml trace element solution [19], was added in 250 mL conical flask. Each flask was inoculated with 2 x 10^8 T. viride spore suspension. Enzyme production was carried out at 30 °C and pH 7.0 in an incubator shaker with a speed of 130 rpm for 96 hours. The culture medium was then harvested by centrifugation at 8000 rpm for 10 minutes at 4 °C. The Clarified supernatant was then used as the source of cellulase. enzyme activity was then determined [20].

Enzymatic hydrolysis

Enzymatic hydrolysis of pretreated slurry was carried out using the method of Holtzapple., *et al.* [21]. 100 g slurry was diluted by the addition of process water to 10% total solids. The diluted slurry was mixed with an appropriate amount of the clarified enzyme (30 FPU g^{-1} of pretreated substrate slurry) in a sterile fermenter containing 0.05 M acetate buffer (pH 5.0). Hydrolysis of the substrates was carried out for 72 hours at 50°C and agitation speed of 100 rpm [22].

Submerged fermentation

Fermentation medium used for this study was composed of carbon source, (Wheat straw hydrolysate), supplemented with KH_2PO_4 (0.5 gL^{-1}); $ZnSO_4 \cdot 7H_2O$ (0.05 gL^{-1}); $MgSO_4.7H_2O$ (0.3 gL^{-1}); $CaCO_3$ (30 gL^{-1}); NH_4NO_3 (2 gL^{-1}) (Huang., *et al.* 2008). The agitation speed was maintained at 180 rpm [23].

Fermentation Procedure

Wheat straw hydrolysate from above was supplemented with required nutrients. The pH was adjusted using 1 N of HCl and 2 M of NaOH pH 5. Thereafter, 10% inoculum size was aseptically added and medium was covered. The medium was then incubated at 35°C for seven days [24]. Fermentation medium was then centrifuged and supernatant was analyzed for lactic acid.

Chemical analysis methods

The reducing sugar content and lactic acid produced were determined using DNS assay method. Lactic acid was determined using High performance liquid chromatography (HPLC) with a C_{18} column and IR detector. Sulfuric acid at 0.7 ml min^{-1} was used as mobile phase. The detection was carried out at 210 nm [25].

Acid-soaking of fresh poultry meat

Lactic acid was diluted using distilled water to achieve desired concentrations (1%, 2% and 3%). The various acid concentrations were then used to soak the previously prepared meat samples, meat samples were also soaked in distilled water under similar conditions and used as control. Treated samples were packed in HDPE film and stored in a refrigerator at 4°C for a period of 14 days [26].

Variation of meat soaking parameters

Meat soaking parameters were varied according to the method of Xiaowei., *et al.* [27]. Different acid soaking-time (5, 10, 15, 20 and 25 minutes) and acid-soaking temperature (10, 20, 30, 40 and 50°C) were evaluated for their effect on the pH of treated meat before and during storage.

Meat acid activity determination

Ten (10) grams of treated meat samples were homogenized in 90 ml of distilled. The mixture was then centrifuged after which the supernatant was collected and titrated using NaOH standard solution (0.001 mol L^{-1} concentration) [28].

Microbiological quality of treated meat samples

The microbiological quality of the treated and untreated poultry meat samples was monitored by culture-dependent methods using plate counts. Total viable counts (TVC), and Coliform counts were determined daily for 14 days according to the methods reported by Yang., *et al.* [29]. 10g of meat sample was aseptically plated onto appropriate agar medium and incubated at 37°C. Plate count agar and violet red bile glucose agar (VRBGA) were utilized for TVC and TCC determination respectively.

Determination of Total Volatile Basic Nitrogen (TVB-N) and Thiobarbituric Acid Reactive Substances (TBARS)

TVB-N was determined according to the procedures described by FAO, [30]. Meat samples were distilled into 2% boric acid solution and titrated with 0.1 N H_2SO_4 (titer). TVB-N (mg N $100g^{-1}$ flesh) was then calculated using the formula:

TVB-N = 14 × (titer-blank).

TBARS was determined according to the method of Schmedes and Hølmer, [31]. Meat samples were mixed with 25 mL of 20%

(w/v) trichloro-acetic acid and filtered. The filtrate was then incubated with aqueous thiobarbituric acid, after which, the absorbance was measured at 532 nm using UV- spectrophotometer. TBARS estimates were expressed as mg malondialdehyde (MDA) kg^{-1} of broiler fillet sample.

Sensory evaluation of treated poultry meat samples

Sensory evaluation of the treated meat samples was carried out at the Department of Microbiology, Federal university of technology, Akure Ondo State, Nigeria. The sensory attributes evaluated includes: appearance, viscosity, texture, colour and odour of the meat samples. The evaluation was carried out on a total of four treatments, by a twelve (12) member semi-trained panel, using a 5-point hedonic scale [32]. The scale ranges from 1 – 5 with details given in table 2.1 below. The twelve member panelists were carefully drawn from members of the university community comprising of students and staff. The panelists were made up of seven females and five males all between the ages of 20 to 50. The panelists were familiarized with the questionnaire of the sensory evaluation; they were then required to evaluate each sample separately without comparison. A minimum total score of 17.0 was considered fresh 12.0 was considered the lowest acceptable threshold.

Scale	Ranges of score	Level of acceptability
1	1.00 - 1.49	Not Acceptable (NA)
2	1.50 - 2.49	Slightly Acceptable (SA)
3	2.50 - 3.49	Moderately acceptable (MA)
4	3.50 - 4.49	Acceptable (A)
5	4.50 - 5.00	Highly Acceptable (HA)

Table 1: Five-point hedonic scale and range of scores.

Statistical analyses

All analyses were performed in triplicates after which the results were presented by means with standard deviation. Data were displayed as mean values attached with the standard deviation. Duncan's new multiple range test ($P < 0.05$) was employed for the determination of significant differences between means, using the SPSS 20 statistics software (IBM, Chicago, Ill., U.S.A.).

Results and Discussion

Properties and yield of all fermentation parameters involved in l-latic acid production

Table 3.1 shows the cellulase activity of crude enzyme obtained from *T. viride,* FPase was 6.25 U/ml, Endoglucanase activity was 8.5 Uml^{-1} and b-glucosidase activity was 5.0 Uml^{-1}. On-site celluloses produced by *Trichoderma viride* was found to effectively hydrolyze available cellulose fractions in wheat straw. This result was in agreement with the findings of Zhao., *et al.* [20].

Figure 1 and Table 2 shows the retention time and peak representing the products generated (L-lactic acid and Bioethanol) from *R. arrhizus* fermentation. L-lactic acid was identified at 4.093 minutes with a peak area of 1026.927, while bioethanol which is a by-product of lactic acid fermentation was also identified. Lactic acid yield obtained as shown in table 3.3 was 14.53 gl^{-1} which cumulated to 41.70% (w/w). This results contrast with the findings of Tanyildizi., *et al*. [33]. They reported lactic acid from immobilized *Rhizopus oryzae* with a value of 96.56 gl^{-1} and percent yield of 64.4%. Taleghani., *et al.* [34] reported a value of 20.5 g/l with a higher yield of 62.5% from whey using lactobacillus. The higher yield of lactic acid in their study could be attributed to the use of better adapted strains of *R. oryzae* and *Lactobacillus* which facilitated higher sugar to acid conversion rates. Ozen and Ozilgen [35] also noted that low enzymatic activity as recorded in this study, is capable of limiting saccharification process which in turn could affect overall yield of lactic acid. The high pKA of lactic acid, its potential as a pH regulator and its antibacterial activity makes it a good preservative agent [36].

Over the years, low cost agro residues have been effectively utilized in the production of lactic acid by *Rhizopus oryzae* through SmF [6]. Wheat straw is an interesting biomass with abundance of cellulose and hemicellulose which can be converted to lactic acid with different organisms through co-fermentation strategies [37].

Parameter	Activity (Uml^{-1})
Filter paper activity	6.5
Endoglucanase activity	8.5
b-glucosidase activity	5.0

Table 2: Cellulase activity of crude enzyme obtained from *T. viride.*

Product	Retention time	Area
L-Lactic acid	4.093	1026.927
Ethanol	7.816	296.136

Table 3: HPLC properties of products identified.

Parameter	Yield
Reducing sugar Yield	34.85 gl^{-1}
Lactic acid Yield	14.53 gl^{-1}
% Yield	41.70

Table 4: Fermentation yield for l-lactic acid production from wheat straw.

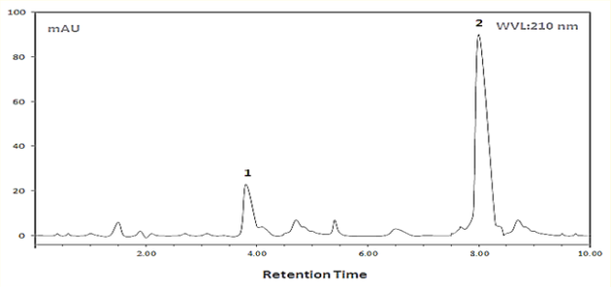

Figure 1: HPLC chromatogram for Rhizopus arrhizus production of lactic acids from Wheat straw.
Key: 1 = Ethanol, 2 = Lactic acid.

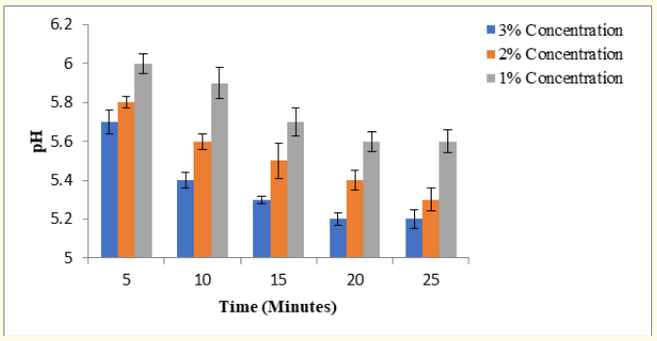

Figure 2: Effect of lactic acid soaking time on the initial pH of poultry meat.

Effect of acid-soaking time and temperature

The effect of soaking time and temperature on the initial pH of the meat samples was estimated using varied acid treatment concentration (1%, 2%, 3%) (Figure 2 and 3). The acid-soaking time was positively related to the initial meat pH. It was observed that increase in the acid-soaking time resulted in a corresponding decrease in the initial pH of the meat samples. Lowest pH of 5.2 ± 0.20, 5.3 ± 0.20 and 5.6 ± 0.10 was observed after 20 minutes of soaking with 3%, 2% and 1% lactic acid solution respectively. This was regarded as the optimum soaking time. The acid-soaking temperature was also positively related to the initial pH of the meat samples up to 30 °C. Further increase in temperature beyond 30 °C led to a rise in the meat pH and hence reduced acidity, this was due to the fact that the organic acids used were highly volatile at temperatures above 30 °C, hence drastically reducing their effectiveness [38]. Lowest pH of 5.1 ± 0.10, 5.2 ± 0.20 and 5.5 ± 0.10 was observed at 30°C soaking temperature with 3%, 2% and 1% lactic acid solution respectively. This was also regarded as optimum. Pre-storage conditions of meat have been identified as one of the major factors that influence the keeping quality of the meat samples. Food processors have resulted to salting, drying etc. in a bid to achieve optimal pre-storage conditions in meat [26]. Varying the acid-soaking time and temperature of the meat treatment, had significant effect on the initial pH of the meat samples ($P < 0.05$). The observed decrease in pH at optimal soaking parameters of 25 minutes and 30 °C respectively enabled the establishment of ideal pre-storage conditions in the meat samples. Reduction in pH (which is a major factor influencing microbial growth) to unfavorable levels have been found to directly improve the keeping quality of most food substances [39]. It was noted that meat treatment with 2% lactic acid concentration had the greatest effect on the pH reduction of meat prior to storage.

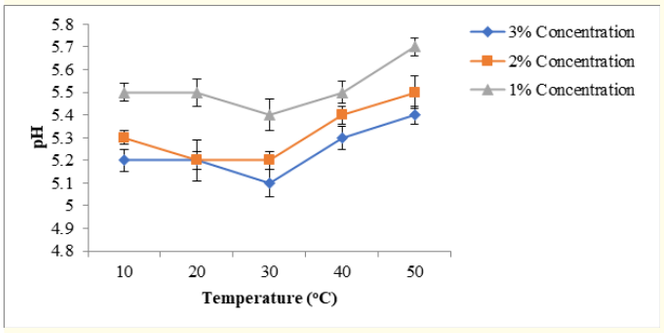

Figure 3: Effect of lactic acid soaking temperature on the initial pH of poultry meat.

Microbiological quality of meat (Total viable count (TVC) and Total coliform count (TCC))

The shelf life and safety of the preserved poultry meat were evaluated by estimating the TVC and TCC of the meat treated with different acid concentrations (1%, 2%, and 3%) at 25 minutes acid soaking-time and 30°C acid soaking-temperature for fourteen days (Figure 4 and 5). Reduction in TVC was directly proportional to the acid treatment concentration used. Highest counts were observed in the control samples, in which the TVC was observed to increase during storage. Lowest TVC of 1.98 ± 0.22 $\log_{10}$ cfu g^{-1} was observed on day 8 of storage, using 3% concentration. However, TVC in all samples was observed to increase after day eight of storage. There were significant differences in the TVC of the meat samples treated with different acid concentration ($P < 0.05$). TCC was also lowest when 3% lactic acid concentration was used. Highest coliform counts were recorded in the control samples, which showed a steady increase in coliform bacteria during storage with highest TCC of 3.11 ± 0.05 $\log_{10}$ cfu g^{-1} on day 14. Lactic acid treatment resulted in considerable reduction in TCC during storage, lowest TCC of 0.04 ± 0.01 $\log_{10}$ cfu g^{-1} was observed on day seven using 3% acid concentration. This was similar to the work of Tian., *et al.* [40], who employed lactic acid in the treatment of beef. An increase in TCC in all samples was observed after day 10. There were significant differences in the TCC of the meat samples during storage with different treatments ($P < 0.05$). TVC and TCC are often regarded as direct quality indicators in food samples and have been proven to have

a positive correlation to food spoilage process and food safety respectively [36]. Initial bacterial count in meat samples were within acceptable range, indicative of proper meat handling/hygiene [41]. TVC and TCC values of 6.0 and 2.0 $\log_{10}$ cfu g^{-1} is regarded as the threshold for fresh meat acceptability by the International Commission on Microbiological Specifications for Foods [42], hence, rendering the control samples completely unacceptable beyond day three. The observed reduction in the rise in TVC and TCC within the acid treated samples as storage progressed was significantly influenced by acid treatment concentration. This reduction in pH can severely affect the growth and survival of non-acidophilic bacteria, which are the group predominantly responsible for meat spoilage and infection [40]. This effect can be attributed to a disruption in pH homeostasis, which is highly critical in microbial metabolism; due to its role in maintaining proper function of biological macromolecules as well as maintaining the kinetic and thermodynamic force of chemical reactions involving protons as metabolites [39]. Although, lactic acid exhibited good bacteriostatic and bactericidal effects on treated meat samples, treatment with a minimum of 2% concentration gave the best result. Ouattara., *et al.* [43], suggested that lactic acid had high bacteriostatic effect due to its pKa value of 3.8, which showed less dissociation than other organic acids, hence making it more lethal to bacteria.

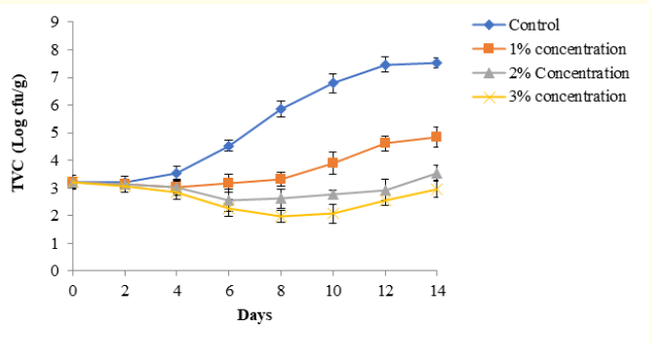

Figure 4: Total viable count of poultry meat treated with different concentration of lactic acid and stored for 14 days.

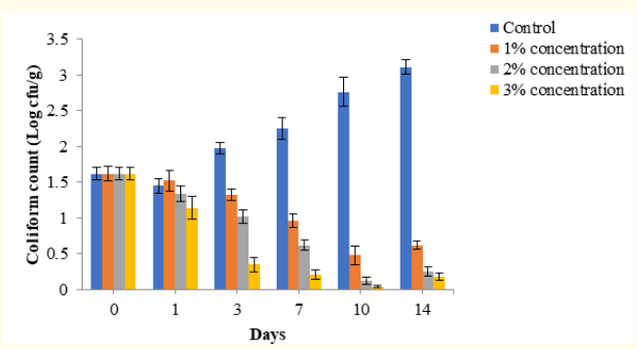

Figure 5: Total coliform count of poultry meat treated with different concentration of lactic acid and stored for 14 days.

Changes in pH and acidity activity of samples during storage

Figure 6 shows the changes in pH of poultry meat stored for 14 days using different concentrations of lactic acid at 25 minutes soaking time and 30°C soaking temperature. The enduring effect of the acid treatments process on the meat samples during storage was further confirmed by the observed changes in pH in both the treated and untreated meat samples. General decrease in the pH of treated meat samples was observed up until day 8, this was contrary to the findings of Han., *et al.* [44] who recorded fluctuations in the pH of broiler meat spread with lactic acid. Lowest pH of 4.8 ± 0.10 was observed using 3% lactic acid concentration at day 12. The pH of the control sample on the other hand was observed to increase as storage progressed beyond day 12 and this can be attributed to the observed increase in microbial growth at this stage of storage [6]. There were significant differences in the pH of the meat samples treated with varying concentrations of acid as storage progressed ($P < 0.05$). Higher acid treatment concentrations led to lower meat pH during storage. The pH of meat plays a vital role in its quality and shelf-life during storage, and any deviations from the acceptable range can result in adverse effects on the color/appearance and water-holding capacity of fresh meat [44]. pH fluctuations of broiler meat during storage have been found to greatly affects the production of sulfur-containing and carbonyl volatiles. Extreme alkaline and acidic conditions in meat during storage greatly increase the production of these volatiles. At these extreme conditions, darkening tend to occur resulting in meat discoloration and loss of flavor [45]. Optimal pH for the preservation of poultry meat was between 4.5 and 5.5, it was however evident that meat treatment with 2 to 3% lactic acid solution led to delayed glycolysis and effectively prevented rapid meat acidification at the early stages of storage; these treatments were also observed to efficiently prevent undesirable pH fluctuations in the treated meat samples at the mid and late stages of storage [46].

Correlation between the meat acid activity, TVC and TCC is shown in figure 7. The higher the meat acid activity, the lower the TVC and TCC, lowest TVC of 1.95 ± 0.08 $\log_{10}$ cfu g^{-1} was observed at an acid activity of 0.4%, while lowest TCC of 0.03 ± 0.01 $\log_{10}$ cfu g^{-1} was observed at same acid activity. Reduction in TVC and TCC during storage showed significant differences in comparison with the acid activity of the treated meat samples ($P < 0.05$). Since the acid activity of the meat samples was a cumulative effect of all treatment parameters, it was conceivable that the observed increase in antimicrobial activity in the form of TVC and TCC reduction, was a direct effect of the use of optimal treatment conditions (in relation to treatment time and temperature) [36].

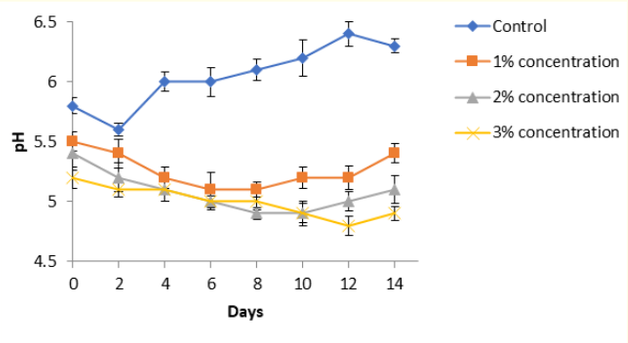

Figure 6: pH changes of poultry meat preserved with different concentration of lactic acid for 14 days.

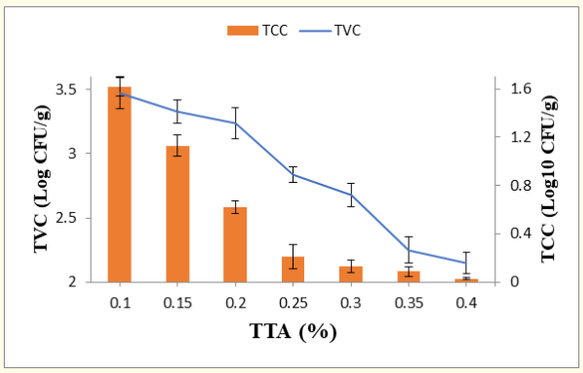

Figure 7: Relationship between acid activity, TVC and TCC of poultry meat during preservation.

Anti-oxidative effect of lactic acid treatment on poultry meat samples

Table 3.4 shows the effect of different concentrations of lactic acid treatments on the formation of Total Volatile Basic Nitrogen (TVB-N) and Thiobarbituric Acid Reactive Substances (TBARS) in poultry meat samples during storage. TVB-N and TBARS content in chicken as an important reference index has been used to evaluate its freshness [47]. TVB-N compounds in chicken contain ammonia, trimethylamine (TMA) and dimethhylamine (DMA), and the level of TVB-N compounds increase as with spoilage by either bacteria or enzymatic degradation. TBARS is an index of lipid oxidation, measuring malondialdehyde (MDA) content, which is one of the degradation products of lipid hydro peroxides formed through oxidation of unsaturated fatty acids [48].

It was clear that there were no significant differences in both TVB-N and TBARS between treatments ($P < 0.05$) on day zero. A significant increase with storage time ($P < 0.05$) in both parameters was observed in all treatment groups. This was in agreement with Alasnier., *et al.* [49]; Rukchon., *et al.* [50] and Rahman., *et al.* [51] who recorded similar differences as storage progressed. Significant differences ($P < 0.05$) were again observed between control and lactic acid treatment groups. The control groups showed the overall highest values with a maximum of 59.37 ± 2.92 mg $100g^{-1}$ TVB-N and 1.95 ± 0.02 mg kg^{-1} TBARS on day 14. An overall reduction in the formation of both TVB-N and TBARS was observed in the treated meat samples during storage as compared to the control (untreated) samples. 3% lactic acid treatment had the highest effect in TVB-N and TBARS reduction with 34.38 ± 0.32^{a} mg $100g^{-1}$ and 0.83 ± 0.01^{a} mg kg^{-1} of meat samples on day 14 respectively. A clear relationship was observed between the microbiological quality on the treated meat samples and the levels of TVB-N and TBARS formation, this was again in agreement with Smaoui., *et al.* [52] who noted that reductions in TVC resulted in similar reductions in the formation of TVB-N. Since TVB-N is a function of protein breakdown, the observed increase may be attributed to the formation of ammonia, which could be a result of residual microbial activity in the meat samples during storage [53]. TVB-N values of all treated groups were below the limit of 40 mg $100g^{-1}$ recommended by FAO [30] at day 14 of storage with the exception of the control which had 59.37 ± 2.92 mg $100g^{-1}$ on day 14 of storage. On the other hand, only 3% lactic acid treatment groups had TBARS values (0.83 ± 0.01a mg kg^{-1}) below the permissible limit of 0.9 mgMDA kg^{-1} recommended by the United States Department of Agriculture [30].

Test	Treatment	Day 0	Day 3	Day 7	Day 10	Day 14
TVB-N (mg $100g^{-1}$)	Control	9.73 ± 1.41a	15.21 ± 1.82b	29.82 ± 1.02d	42.59 ± 1.61f	59.37 ± 2.92g
	1%	9.73 ± 0.41^{a}	12.96 ± 1.00^{a}	19.83 ± 1.00^{b}	32.46 ± 0.79^{c}	42.55 ± 1.08^{c}
	2%	9.73 ± 0.41^{a}	11.21 ± 1.00^{a}	16.30 ± 1.00^{b}	25.51 ± 0.79^{c}	37.46 ± 1.08^{a}
	3%	9.73 ± 0.41^{a}	10.83 ± 0.63^{a}	15.10 ± 0.10^{b}	23.46 ± 1.03^{b}	34.38 ± 0.32^{a}
TBARS (mg kg^{-1})	Control	0.18 ± 0.03^{a}	0.49 ± 0.02^{c}	0.92 ± 0.09^{d}	1.53 ± 0.07^{e}	2.01 ± 0.02^{d}
	1%	0.18 ± 0.03^{a}	0.39 ± 0.02^{b}	0.62 ± 0.02^{b}	0.79 ± 0.01^{b}	0.98 ± 0.04^{b}
	2%	0.18 ± 0.03^{a}	0.35 ± 0.02^{a}	0.50 ± 0.06^{b}	0.73 ± 0.09^{b}	0.91 ± 0.08^{b}
	3%	0.18 ± 0.03^{a}	0.29 ± 0.05^{a}	0.46 ± 0.07^{a}	0.64 ± 0.06^{a}	0.83 ± 0.01^{a}

Table 5: Changes in TVB-N and TBARS content of poultry meat treated with different concentration of lactic acid and stored for 14 days.

Data are represented as mean ± standard deviation, n = 3 with the same superscript down the column are not significantly different ($P = 0.05$).

Treatments	Day	Appearance	Viscosity	Texture	Odour	Color	Total
Control	3	4.50 ± 0.05^{a}	3.64 ± 0.02^{b}	4.05 ± 0.01^{ab}	4.12 ± 0.03^{a}	4.00 ± 0.00^{ab}	20.31
	7	2.36 ± 0.03^{c}	2.05 ± 0.01^{cd}	1.81 ± 0.01^{d}	1.28 ± 0.04^{d}	2.35 ± 0.10^{c}	9.85
	10	1.93 ± 0.01^{dc}	1.26 ± 0.06^{d}	1.28 ± 0.04^{d}	1.30 ± 0.05^{d}	2.04 ± 0.02^{cd}	7.81
	14	1.49 ± 0.02^{d}	1.02 ± 0.02^{d}	1.05 ± 0.01^{d}	1.10 ± 0.10^{d}	1.86 ± 0.03^{d}	6.52
1% lactic acid treatment	3	4.50 ± 0.25^{a}	3.90 ± 0.17^{b}	4.20 ± 0.10^{ab}	4.12 ± 0.21^{a}	4.00 ± 0.00^{ab}	20.72
	7	4.06 ± 0.13^{c}	2.76 ± 0.26^{cd}	2.84 ± 0.14^{d}	2.30 ± 0.15^{d}	2.74 ± 0.21^{c}	14.70
	10	2.94 ± 0.28^{dc}	2.30 ± 0.20^{d}	1.52 ± 0.26^{d}	1.87 ± 0.24^{d}	2.44 ± 0.22^{cd}	11.07
	14	2.10 ± 0.20^{d}	1.53 ± 0.13^{d}	1.48 ± 0.18^{d}	1.13 ± 0.06^{d}	1.81 ± 0.17^{d}	8.05
2% lactic acid treatment	3	4.90 ± 0.00^{a}	3.77 ± 0.02^{b}	4.14 ± 0.10^{a}	4.30 ± 0.10^{a}	4.26 ± 0.13^{a}	21.37
	7	4.21 ± 0.01^{a}	3.48 ± 0.05^{b}	3.72 ± 0.02^{b}	2.98 ± 0.13^{c}	3.78 ± 0.03^{b}	18.17
	10	3.25 ± 0.06^{b}	3.56 ± 0.09^{b}	3.21 ± 0.01^{b}	2.44 ± 0.14^{c}	2.02 ± 0.01^{ab}	14.48
	14	2.58 ± 0.07^{c}	2.71 ± 0.16^{c}	2.25 ± 0.09^{d}	1.93 ± 0.02^{d}	2.63 ± 0.01^{c}	12.10
3% lactic acid treatment	3	3.84 ± 0.00^{a}	4.03 ± 0.03^{ab}	4.20 ± 0.10^{a}	4.38 ± 0.09^{a}	3.91 ± 0.07^{a}	20.36
	7	3.80 ± 0.12^{a}	3.83 ± 0.10^{b}	3.90 ± 0.08^{b}	3.10 ± 0.05^{b}	3.55 ± 0.10^{a}	18.18
	10	3.09 ± 0.04^{b}	3.17 ± 0.02^{b}	2.84 ± 0.03^{c}	2.88 ± 0.01^{c}	1.97 ± 0.06^{c}	13.97
	14	2.91 ± 0.10^{c}	2.85 ± 0.05^{c}	2.31 ± 0.03^{c}	2.12 ± 0.02^{c}	1.52 ± 0.10^{c}	11.71

Table 6: Sensory parameters of poultry meat preserved with different concentration of lactic acid for 14 days.

Data are represented as ± standard deviation, data with same superscript down the column are not significantly different ($P < 0.01$).

Effect of acid treatment on sensory parameters

The sensory parameters of untreated and treated poultry meat with different concentration of lactic acid (1%, 2%, and 3%) at 25 minutes soaking time and 30°C soaking temperature for 14 days is shown in Table 3.5. There were significant differences in sensory parameters of meat samples treated with varying acid concentrations ($P < 0.05$). Lactic acid treatment had positive effects on the sensory parameters of poultry meat when compared with the control (untreated) samples. There were no significant differences between the treatments and control samples at day 3 ($P < 0.05$); all samples maintained freshness, and this was an indication of the ability of lactic acid treatments to extend poultry meat shelf-life, without adversely affecting its sensory quality [54]. On the other hand, significant differences ($P \leq 0.01$) were observed in the sensory parameters of treated and untreated meat samples, as well as between different treatment concentrations as from day seven. Only samples treated with 2% acid concentration and above were able to maintain freshness at day seven, [32]. As at day 14, only meat samples treated with 2% lactic acid (12.10 score) was acceptable by the panelist; although they were no longer fresh, they had scores above 12.00. treatment with concentrations above 2% was observed to adversely affect the colour and appearance of the meat samples. The maintenance of sensory parameters observed in the acid treated groups were probable due to one or more carboxylic acid or acid phenolic groups present in the treatment acids such as; amides, esters and peptides [55]. These carboxylic groups play a functional role in lipids and protein metabolism and acid-base balance, thereby positively influencing the sensory parameters of poultry meat [45]. These findings were in accordance with Bobko., *et al.* [56], who found significant positive influence of different plant supplement containing organic acids on the sensory quality of poultry meat.

Conclusion

Lactic acid produced from the fermentation of wheat straw with *R. arrhizus* significantly inhibited the proliferation of spoilage organisms as well as the rate of protein and lipid oxidation in treated meat samples. It was found that untreated poultry meat had a maximum shelf-life of 4 days at 4°C, while poultry meat treated with at least 2% lactic acid was preserved for up to 12 days. The presence of one or more carboxylic acid or acid phenolic groups present in lactic acid such as; amides, esters and peptides makes it an efficient meat preservative agent. This study gives insight into further industrial application of lignocellulosic biomass with emphasis on food preservation. However, less expensive conversion and purification techniques should be explored in order to make the whole process more feasible.

Acknowledgments

Special appreciation goes to the entire staff of the Department of Microbiology, School of life sciences, federal university of technology Akure, for the facilities and assistance provided to accomplish the present research.

Conflict of Interests

The authors declare no conflict of interest.

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