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Antimicrobial Effect of Juice Extract From Fermented Cabbage Against Select Food-Borne Bacterial Pathogens

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Abstract: As part of ongoing efforts to use natural preservatives in meat preservation, this study investigated the antimicrobial effect of fermented cabbage juice against select food-borne bacterial pathogens. Unfiltered and filtered fermented cabbage (*Brassica oleceracea* L. var. *capitata*) juice were used for susceptibility testing using the dilution and agar well diffusion techniques. The screening was done against four standard bacterial strains (*Salmonella enteritidis* 13076, *Escherichia Coli 25922*, *Staphylococcus aureus* 43300 and *Staphylococcus aureus* 29213) and seven clinical isolates (*Escherichia coli*, *Vibrio cholerae*, *Salmonella typhi*, *Salmonella typhimurium, Escherichia coli* 0157; H7, *Staphylococcus aureus* and *Salmonella enteritidis*) of food borne bacterial pathogens. Pathogens grew well in juices from cabbage that was fermented for 28 days but were inhibited to varying extents in unfermented and fermented juices for up to 21 day cabbage fermentation. Activity was more pronounced against gram negative than gram positive bacteria. Inhibition was reduced, when the juice was filtered with 0.2 µm filter following centrifugation. The findings suggest that filtration and fermentation time could be factors responsible for activating a precursor into an inhibitory compound. It is concluded that fermented juice might be more effective against food-borne pathogens and spoilage bacteria when applied unfiltered and at day-21 ferments in meat.

Key words: *Brassica oleracea*, Fermentation, Antimicrobial activity, Bacterial pathogens.

INTRODUCTION

The antibacterial activity of cabbage juice has been of interest to researchers with the activity been reported to be due to the glucosinolates degradation by-products found in the juice[1,2]. Cabbage juice has been shown to have antibiotic and antifungal activity against a wide range of bacteria, and has been used traditionally in the treatment of lung diseases[3,4]. Such activity has recently caused the need to revisit the potential of plant products as raw ingredients in the processing of other foods[5,6]. Cultivated plants with antibacterial activity would in general have more economic potential due to their grater adaptability to commercial propagation. Fermentation of the cabbage juice could enhance the bacteriostatic effect of the cabbage juice as acidified sodium chloride may be formed from the ferment[7]. Such juice may have potential for extended use in the biopreservation of other foods. There is however limited information on the antimicrobial activity of fermented cabbage juice extracts to further gauge its possibility as a bio-preservative. Therefore, in this study, the antimicrobial effect of fermented cabbage juice was determined against selected microbial pathogens. This was to gain a better understanding of the bio-preservative possibilities of the extracts is to be considered for harness in food systems.

MATERIALS AND METHODS

Fermentation of Cabbage and Juice Extraction: Fresh cabbage heads were randomly sourced from three sites markets in Kenya. The cabbages were shredded using a Black and Decker (Model FX750, Shelton, CT) Food Processing unit. Five hundred grams were weighed and common salt (Nacl) added at 2.25 %. The cabbage shreds were stacked in fermentation bottles in triplicate and left to ferment spontaneously at room temperature (approximately 20^{o} C). Juice samples were taken at day 0, 7, 14, 21 and 28 of fermentation for the inhibition experiments. For trials with filtered juices a 0.2 µm filter was used.

Bacterial Strains and Culture Conditions: Eleven microbial strains were selected on the basis of their importance as food pathogens. Gram positive and gram

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negative bacterial pathogens were obtained from culture collection maintained by the Kenya Medical Research Institute (KEMRI), Nairobi Kenya. Overnight cultures were prepared by transferring a loop full of stock cultures to tube having nutrient agar and incubating at 35^0C for 24 h. Isolated colonies were used to prepare a bacterial suspension of 0.5 McFarland Standard 1 containing approximately 10^8 cfu/ ml. These cultures were then used as inoculums for culturing pathogens on petri dishes for the antimicrobial test. Water was used as negative control and ampicilin was used as positive control. Each sample was done in triplicate. Antibacterial activity was evaluated by quantifying zones of inhibition of bacterial growth after 24 hrs.

Screening for Antibacterial Activity / Susceptibility Testing: The screening for antibacterial activity and susceptibility testing was done as described by Saeed & Tariq [1]. In brief, nutrient agar (NA) (Oxoid) was used as base medium for screening of antibacterial activity and tryptic soy broth (TSB) (Oxoid) for the preparation of inoculum. McFarland tube number 0.5 was prepared by mixing 9.95 ml of 1% (vol/vol) sulphuric acid and 0.05 ml of 1.175% barium chloride in distilled water in order to estimate bacterial density. This McFarland preparation gave a bacterial density of 10^8 cfu/ mL. The tube was sealed and used for comparison of bacterial suspension with the standard whenever required.

Quality Control of Media and Reagents: Growth medium was prepared according to the manufacturer's instructions. After autoclaving, the medium was cooled to 50°C. About 25ml of medium per plate was measured into 15 x 100-mm plastic petri dishes on a level pouring surface to a uniform depth of 4 mm. Freshly prepared plates were used the same day or stored in a refrigerator (2° to 8°C) for up to 2 weeks. If plates were not used within 7 days of preparation, they were wrapped in plastic to minimize evaporation. Just before use, any excess moisture on the surface was removed by placing plates in an incubator (35° to 37°C) until the moisture evaporated (about 10 to 30 min). Each new lot was quality controlled before use by testing the *E. Coli* ATCC 25922 or *Staphylococcus aureus* ATCC 25923 standard strains, used with every test run for *Enterobacteriaceae* and gram-positive aerobes, respectively. Zone diameters obtained for ATCC 25922 were compared with the National Committee for Clinical Laboratory Standards (NCCLS) published limits [8].

Screening of antibacterial activity was performed by the well diffusion technique[1]. The nutrient agar plates were seeded with 0.1 mL of the standardized inoculum of each test organism. The inoculum was spread evenly over the plates with a sterile glass spreader. A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the nutrient agar.

Turbidity of the inoculum suspension was adjusted by holding the test tubes against a white background with contrasting line. A sterile cotton swab was dipped into the suspension used to pick colonies. The swab was streaked over the entire surface of the medium three times, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculum. About 200 µL of each juice of fermented cabbage was introduced in the well and allowed to stand for 15 minutes before incubation.

The inoculated plates were incubated at 37 ^{o}C for 24 hours. After incubation, the diameter of the zones of complete inhibition were measured (including the diameter of the disk) and recorded in millimetres (mm). The scale of measurement was chosen as follows: ≥ 26 mm. Zone of inhibition - strongly inhibitory; 1- 26 mm- moderately inhibitory; 11- 16 mm mildly inhibitory; < 11mm inhibitory to none inhibitory.

The working supply of antimicrobial disks stored in the refrigerator (4°C) was used as positive control to evaluate cultures for possible antibiotic resistance patterns that would have affected the sensitivity of assay. Microbial sensitivity disks (Oxoid, UK) for the drug resistance patterns used were gentamicin (G) (10 μg/disk), ciprofloxacin (Cp) (5 μg/disk) and nalidixic acid (Na) (30 μg/disk). Upon removal of the disks from the refrigerator, the package containing the cartridges was left unopened at room temperature for approximately 1 hour to allow the temperature to equilibrate to room temperature before using. This reduced the amount of condensation on the disks. The disks were placed individually with sterile forceps, and then gently pressed down onto the inoculated agar. After the disks were placed on the plate, the plates were inverted and incubate at 37°C for 18 - 24 hours. The distance from the colonies closest to the disk centre was measured and then doubled to obtain a diameter.

Minimum Inhibitory Concentrations of The Juices: The serial dilution technique was used to test levels of diluted juice that could cause inhibition to selected pathogens. Juices were serially diluted using sterile distilled water to levels of up to 1: 10^6. The wells so formed on pathogen inoculated plates were filled with diluted juices on plates at portions of 0.2 ml then incubated at 37 ^{0}C for 24 hrs. Plates were then observed for inhibition zones up to the lowest dilution where inhibition occurred. This dilution was recorded as the minimum inhibitory concentration.

The Growth Experiment: Juices were added at replacement values of 0%, 25%, 50%, 75%, and 100% respectively to sterile tryptic soy broth, inoculated with 100 µL of 0.5 McFarland standard culture containing 10^8 cfu/ mL of organisms approximating to a culture of 10^7 cfu/ mL. This was incubated for 24 hrs at 37^0C. After incubation sample turbidity was measured using a spectrophotometer and absorbance readings recorded for each sample. A sample of 100% represented microbial growth on juice alone and 0% represented growth on media broth without added juice.

Growth Inhibition Experiments: Response surfaces methodology for the combined effect on growth of temperature, day of fermentation and sterile media dilutions with fermented juice were studied at four levels each. The double dilution method was used. About 10 mL of juices from Kinale that were fermented for 0, 7, 14 and 21 days were added to 4 prepared tubes of 10 ml sterile tryptic soy broth to give 0.5, 0.25, 0.125 and 0.0625. The dilutions were inoculated with 0.1 mL of 0.5 McFarland standard culture containing 10^7 cfu/ mL of three standard ATCC bacterial strains. The culture was the incubated at 4^0C, 22 ^{0}C, 37 ^{0}C and 42 ^{0}C respectively. After 24 hr incubation the optical density was recorded to obtain the growth responses.

Trials with Pathogen Contaminated Meat Samples: Lean upper cut meat weighing 2 kg was purchased from a local supermarket. Blocks of meat measuring 2.5 x 2.5 by 1.25 cm were incised from the meat using a knife. Then, 0.1 ml of inoculum was added drop wise to the beef cube and distributed by overturning using a sterile glass spreader. After a 30-min holding period at room temperature to allow cell attachment, the cubes were dipped in 150 ml fermented cabbage juices of day 14 and 28. The dipped cubes were placed under refrigeration at 4 ^{0} C for 4 days. After 4 days the cubes were removed from the juice and washings made using 250 ml of physiological saline. The saline with beef was shaken for 30 s then, 1 ml aliquots of the wash was inoculated on spread plates of selective agar, incubated at 37 ^{0}C and growth observed after 24 hr.

Statistical Analysis: Experiments were performed in triplicate and diameters of zones of inhibition were expressed as mean ± standard deviation. Where the main effect was significant ($P < 0.05$) means were separated using the least significant difference (LSD) test. Response surface methodology was used for experimental design, data analysis and model building using SAS (Version 8.2, SAS Inst. Inc., Cary, N.C., USA) for the combined effect of three hurdles on growth of three microbial strains. Three independent variables vis a vis temperature, day of juice fermentation and level of media dilution with the juice, with four levels for each variable were used, while the independent variable was growth which was taken from absorbance readings. Responses were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The results of susceptibility testing with unfiltered and filtered juices from fermented cabbage are presented in table 1 and fig 4. Both unfiltered and filtered juices showed antimicrobial activity against the tested food-borne pathogens to varying extents with the unfiltered juices having a greater effect. Sulphur compounds from cabbage have been found to show strong antimicrobial activity[9]. Fermentation does not seem to impair the antimicrobial activity suggesting that the activated precursors into inhibitory compounds persist during the process.

The highest activity observed in juices from cabbage fermented for 21 days for all the three locations was observed in the clinical strains of *V. cholerae, S. enteritidis*, and *S. typhi* while the lowest activity was observed in tests with *E. coli* ATCC 25922. This seems to coincide with the known quality values for sauerkraut which has been observed after day 21 fermentation periods[9]. The amounts of fermentable sugars and pH are lowest at this point with highest titratable acidity as percent lactic acid[9]. Moderate to slight inhibition of the pathogens was observed with juices from cabbages fermented for 7 and 14 days respectively indicating that day 7 day ferments showed better effect than day 14 ferments.

The susceptibility of individual bacterial strains to unfiltered juice from fermented cabbage was varied. The descending order of the juice antimicrobial activity was *S. Enteritidis* 13076, *S. aureus* 43300, *S. aureus* 29213 and *E. coli* 25922 for the ATCC strains. A stronger antimicrobial activity of the juice was observed on the clinically isolated strains compared to the laboratory strains. The pathogen most resistant to antimicrobial activity of the studied juices was *E. coli* 25922. This observation was similar to that made with the filtered juices on the same organism. *Escherichia coli* has been shown to offer a higher antimicrobial resistance in other studies compared to other strains [10]. There was a significant difference in the activity of the juice on the individual standard strains studied (table 1). A mild inhibition with mean zones of 13.1 mm, 11.7 mm, 13.9 mm and 12.9 mm for *S. Enteritidis* 13076, *E. coli* 25922, *S. aureus* 43300 and *S. aureus* 29213, respectively were recorded. Inhibitory activity was the same for *S. Enteritidis* 13076 and *S. aureus* 29213 on separation of treatment means. The highest growth inhibition was observed on *S. aureus* and

lowest on *E. coli* ATCC strains. *Staphylococcus aureus* has previously been found to be more susceptible than *E. coli* strains in studies with spices used in meat processing[11].

Inhibition increased with increasing days of fermentation. Juice from cabbage fermented for 21 days was observed to have the highest mean inhibition for all the four ATCC strains irrespective of geographic location, except for day-21 ferments from Dundori which exhibited strongest inhibition on all strains studied. In a different study where several spices were studied only one spice showed highest antimicrobial activity on all strains studied[11]. This inhibition as shown in table 2 was however eliminated in the juice from unfermented cabbage and 7 days fermentation when the juice was filtered for all the organisms.

The results of tests with meat samples are presented in table 3. No growth was observed in the selective media used for growth of the tested strains after 24-48 hrs. This growth inhibition could have been due to a number of antimicrobials in the juice. Lactic acid bacteria in ground meat has been shown in a precious study to inhibit the growth of *E. coli* 0157:H7 in ground beef[13]. The reduction of pH during fermentation has further been shown to cause the protection of ground pork against pathogens[13,14, 15].

In this study while some bacterial strains were inhibited with undiluted juices inhibition was generally observed after dilution of $1:10^3$ on both ATCC and clinical isolates of *S. aureus* strains (Table 3). The results of this study are different from what was observed with cabbage juices in a similar study[16]. However, fermented juices in dilutions of 1:1 and 1:5 showed dose related effects. Allyl isothiocyanates in sauerkraut have been shown to inhibit *Salmonella typhimurium* and *E. coli*[9,17] although this has to be treated cautiously as many factors in food systems including buffering of proteins, emulsifier effects, phospholipids and fatty acids cause masking in the food matrix[18].

The linear dependence of absorbance on the number of microorganisms in CFU/ml was observed in figures 1- 3. Day 7 and 21 showed the highest inhibition to growth. Inhibition to growth was mainly due to the effect of temperature and day of fermentation minimum inhibition being exerted at 37 ^{0}C for *S. enteritidis* at the seventh day of fermentation (table 1). The critical temperature found in this work was being 42 ^{0}C on the 7th day. The findings indicate that for effective antibiotic effect, juice fermentation must proceed beyond 7 days. The level of juice dilution studied were found to cause an insignificant ($p > 0.0001$) effect on growth for this organism.

Table 1: Susceptibility of standard American Type Culture Collection (ATCC) pathogenic bacterial strains to juice from fermented cabbage

Juice type	Inhibition zone diameter in mm (Mean* ± SD)							
	UNFILTERED JUICES				FILTERED			
Site / Day	1	2	3	4	1	2	3	4
D 0	17 ± 4.36^{e}	13.33 ± 1.53^{bcd}	16 ± 3.46^{efg}	13 ± 1.00^{b}	R	R	R	R
M0	16 ± 6.08^{e}	13.66 ± 0.58^{bcd}	12.66 ± 0.58^{h}	13 ± 1.00^{b}	R	R	R	R
K0	16 ± 5.29^{e}	11 ± 0.00^{cde}	17.33 ± 0.58^{def}	12 ± 1.73^{b}	R	R	R	R
D7	17 ± 2.64^{e}	9.33 ± 1.155^{ef}	14.33 ± 1.53^{fgh}	12 ± 2 00^{b}	R	R	R	R
M7	22 ± 5.29^{bc}	14 ± 1.00^{abc}	25 ± 4.36^{b}	17.33 ± 1.15^{a}	13.5 ±2.12^{d}	R	R	12.5 ± 0.71^{c}
K7	16.33 ± 5.85^{e}	10.66 ± 1.53^{def}	17.66 ± 1.53^{de}	12.33 ± 0.58^{b}	R	R	R	R
D14	16 ± 3.61^{e}	R	13.66 ± 0.58^{gh}	12.33 ± 0.58^{b}	13 ± 2.83d	14 ±1.41^{b}	15.5 ± 0.71^{d}	13.5 ± 0.71^{c}
M14	20.33 ± 3.06^{cd}	17 ± 1.00^{a}	20.33 ± 1.53^{cd}	17 ± 1.00^{a}	15.5 ± 0.71c	14 ± 0.00^{b}	20.5 ± 0.71^{bc}	17.5 ± 0.71^{b}
K14	17.66 ± 0.58^{de}	12 ± 0.00^{cde}	17 ± 1.00^{ef}	15 ± 1.00^{ab}	15.5 ± 0.71c	11.5 ± 0.71^{cd}	19.5 ± 0.71^{c}	13.5 ± 0.71^{c}
D21	24 ± 4.58^{ab}	16.33 ± 0.58^{ab}	21 ± 1.73^{c}	17.66 ± 0.58^{a}	17.5 ± 0.71^{b}	19 ± 0.00^{a}	21.5 ± 0.71^{b}	20.5 ± 0.71^{a}
K21	23 ± 2.65^{bc}	17 ± 1.00^{a}	23 ± 1.00^{bc}	18 ± 1.00^{a}	18 ± 0.00 ^{b}	15 ± 0.00^{b}	20.5 ± 0.71^{bc}	17.5 ± 0.71^{b}
M21	27.66 ± 1.52^{a}	R	28.33 ± 1.15^{a}	12.33 ± 0.58^{a}	17.5 ± 0.71^{b}	12 ± 0.00^{c}	16.5 ± 0.71^{d}	18.5 ± 0.71^{b}
D28	10.66 ± 2.31^{f}	R	14.66 ± 1.53^{efg}	12 33 ± 0.58^{b}	17.5 ± 0.71^{b}	14 ±1.41^{b}	15.5 ± 0.71^{d}	12.5 ± 0.71^{c}
M28	R	R	16 ± 4.58^{efg}	R	21.5 ±2.12^{a}	18 ±1.41^{a}	23.5 ± 0.71^{a}	20.5 ± 0.71^{a}
K28	R	R	11.66 ± 0.58^{h}	R	R	R	R	R

* Means of 3 determinations R- Zone diameter 8 ± 0.00 indicating resistance abc - means followed by the same letter along the same column are not significantly different 1- S. Enteritidis 13076 2- E. coli 25922 3- S. aureas 43300 4- S. aureas 29213 D- Dundori M- Molo K - Kinale 0, 7, 14, 21, 28 - Days of fermentation

Table 2: Susceptibility of clinically isolated pathogenic bacterial strains to fermented cabbage juice

Site / Day	Inhibition zone diameter in mm (Mean* ± SD)						
	Strain						
	5	6	7	8	9	10	11
D 0	11 ± 1.41^{cd}	14.4 ± 0.71^{fg}	12.5 ± 0.71^{cd}	R	15 ± 1.41^{abc}	12.5 ± 0.71^{e}	17 ± 0.00^{dcd}
M0	17.5 ± 0.71^{ab}	16.5 ± 0.71^{ef}	12 ± 1.41^{gh}	12.5 ± 0.71^{bc}	14.5 ±3.54^{abc}	13.5 ± 2.12^{de}	18 ± 1.41^{bcd}
K0	11 ± 1.41^{cd}	14.5 ± 0.71^{fg}	13.5 ± 0.71^{efg}	10.5 ± 0.71^{cd}	13 ± 1.41^{c}	14 ± 1.41^{cde}	14 ± 1.41^{de}
D7	19 ± 1.41^{a}	18.5 ± 0.71^{cdef}	14 ± 1.41^{defg}	10.5 ± 0.71^{cd}	14 ± 1.41^{bc}	12.5 ± 0.71^{e}	16.5 ± 0.71^{a}
M7	12 ± 0.00^{cd}	18 ± 1.41^{def}	17 ± 0.00^{cde}	10.5 ± 0.71^{cd}	12.5 ± 0.71$_{c}$	13 ± 1.41^{e}	17.5 ± 0.71^{bcd}
K7	18 ± 1.41^{a}	23.5 ± 0.71^{ab}	18 ± 1.41^{cd}	12.5 ± 0.71^{bc}	18.5 ± 0.71^{a}	17.5 ± 0.71^{dcd}	20 ± 2.83^{b}
D14	18.5 ± 0.71^{a}	22 ± 1.41^{abcd}	21 ± 1.41^{bc}	13 ± 1.41^{bc}	13 ± 1.41^{c}	12.5 ± 0.71^{e}	21 ± 2.83^{b}
M14	17.5 ± 0.71^{ab}	22.5 ± 4.95^{abc}	25 ±1.41b	12.5 ± 0.71^{bc}	13 ± 1.41^{c}	R	19.5 ± 0.71^{b}
K14	13.5 ± 0.71^{bc}	17.5 ± 0.71^{ef}	23.5 ± 0.71^{b}	13.5 ± 2.12^{abc}	14.5 ± 2.12^{abc}	13.5 ± 0.71^{de}	15 ± 1.41^{cd}
D21	19.5 ± 2.12^{a}	20 ± 2.83^{bcde}	24.5 ± 0.71^{b}	13.5 ± 2.12^{abc}	16.5 ± 0.71^{abc}	16.5 ± 0.71^{bcde}	18.5 ± 0.71^{bc}
K21	21 ± 2.83^{a}	25 ± 1.41^{a}	23 ± 5.66^{b}	16.5 ± 0.71^{ab}	18.5 ± 0.71^{a}	18 ± 1.41^{bc}	18.5 ± 0.71^{bc}
M21	18 ± 7.07^{a}	22.5 ± 4.94^{abc}	29.5 ± 0.71^{a}	15.5 ± 0.71^{ab}	14 ± 1.41^{bc}	19.5 ± 0.71^{b}	15.5 ± 0.71^{a}
D28	R	14.5 ± 0.71^{fg}	17 ± 2.83^{cde}	13.5 ± 0.71^{abc}	17.5 ± 1.53^{ab}	16.5 ± 0.71^{bcde}	18 ± 1.41^{bcd}
M28	R	18.5 ± 4.91^{cdef}	16.5 ±2.12^{def}	17.5 ± 0.71^{a}	17.5 ±2.12^{ab}	25 ± 1.41^{a}	19 ± 1.41^{bc}
K28	R	11.5 ± 0.71^{g}	R	10.5 ± 0.71^{cd}	R	R	10 ± 0.00^{e}

* Means of 3 determinations R- Zone diameter 8 ± 0.00 indicating resistance abc - means followed by the same letter along the same column are not significantly different 5- E. coli 6- V. cholerae 7- S. typhi 8- S. typhimurium 9- E coli 0157:H7 10- S aureas 11 -S. enteritidis

Table 3: The canonical analysis for the estimated stationary points and overall curve shapes of the growth responses of the bacterial strains.

Bacterial Strain	Eigen values	Eigen vectors			Stationary point	critical values		
		Day	Dilution	Temperature			Uncoded	Coded
S. enteritidis 13076	0.164	-0.097	0.978	-0.186		Day	8.102	-0.228
	-0.308	0.884	-0.001	-0.467	Saddle point	Dilution	0.106	-0.801
	-0.447	0.457	0.21	0.864		Temperature	34.793	0.621
E. coli 25922	0.179	-0.037	0.91	-0.413		Day	6.039	-0.425
	-0.223	-0.334	0.379	0.863	Saddle point	Dilution	0.316	0.157
	-0.497	0.942	0.17	0.29		Temperature	44.342	1.123
S. aureas 43300	-0.097	-0.173	0.909	-0.379		Day	4.069	-0.612
	-0.275	-0.371	0.297	0.881	maximum point	Dilution	0.728	2.042
	-0.494	0.913	0.293	0.286		Temperature	21.102	-0.099

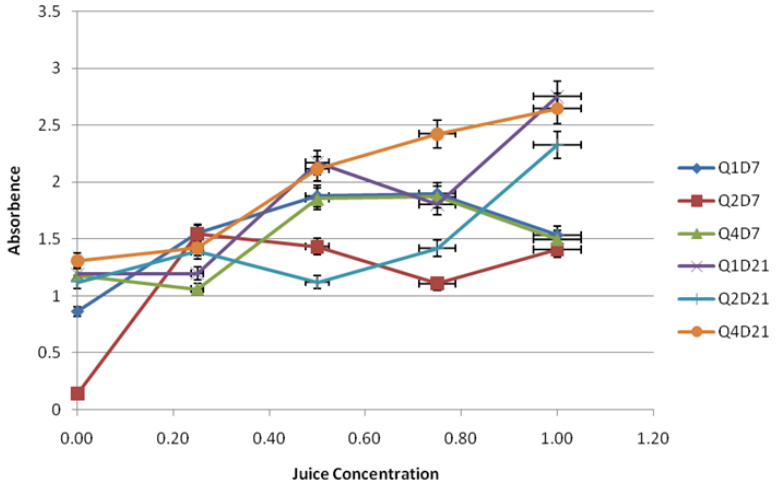

Fig. 1: Scatter diagram for effect of fermented cabbage juice from D (Dundori) on the growth of the three ATCC strains. Q1- *S. Enteritidis* 13076, Q2- *E coli* 25922, Q4- *S aureas* 29213. D7- Day 7 fermentation, D21- Day 21 fermentation.

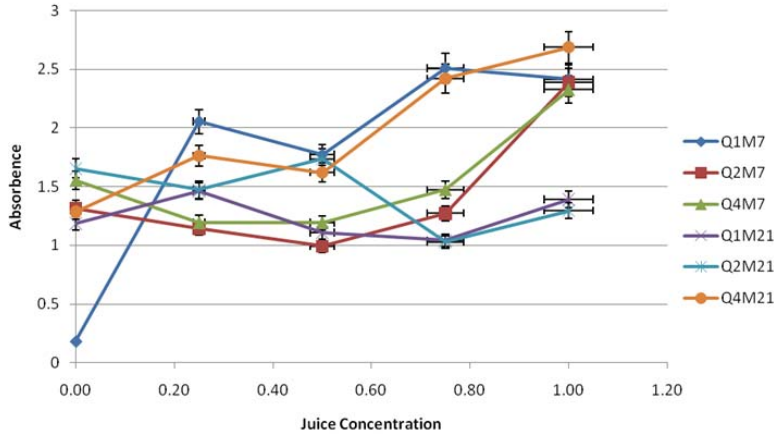

Fig. 2: Scatter diagram for effect of fermented cabbage juice from M (Molo) on the growth of the three ATCC strains. Q1- *S. Enteritidis* 13076, Q2- *E coli* 25922, Q4- *S aureas* 29213. D7- Day 7 fermentation, D21- Day 21 fermentation.

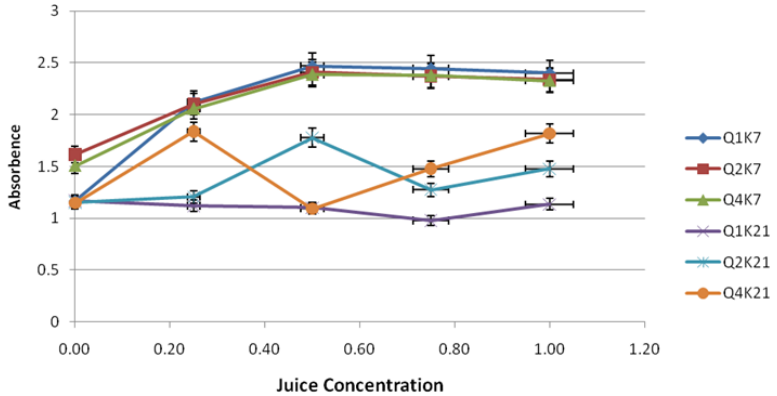

Fig. 3: Scatter diagram for effect of fermented cabbage juice from K (Kinale) on the growth of the three ATCC strains. Q1- *S. Enteritidis* 13076, Q2- *E coli* 25922, Q4- *S aureas* 29213. D7- Day 7 fermentation, D21- Day 21 fermentation.

The day of juice fermentation and incubation temperature from table 1, was observed to significantly ($p< 0.0001$) affect the growth response of *S. enteritidis*. Overall in this study the activity of the juice was found to be dependent on the bacterial strain and temperature of incubation. The organosulfur components methyl methanethiosulfinate and s-methyl -L- cysteine sulfoxide and sulphides have been found in unfermented cabbage to have antimicrobial effects on some strains[9]. These compounds were however not determined in this study but they might be able to persist during the process of fermentation. It has been shown that organic acids found in fermented foods can inhibit a wide range of microorganisms[19,20].

Conclusion: The results of this study indicate that the strongest inhibitory activity was found in fermented cabbage juices after 21-day fermentation. The unfiltered juice extract further had the higher level of inhibition. In optimising the use of the fermented vegetable the juice could thus be best after a 21 day fermentation period and unfiltered for it to be used as a preserving ingredient in meat curing for the studied microbes.

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