EFFECT OF SPONTANEOUS POTATATO (*Solanum tuberosum* L.) FERMENTATION ON SUGARS AND ACRYLAMIDE COMPOSITION OF CRISPS AND FRENCH FRIES

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Abstract
Fermentation is among the oldest methods of food preservation. During fermentation some nutrients are utilized to produce acid and other compounds which help in stability of the fermented products. The aim of this study was to investigate the effect of fermentation on quality of potatoes. Five potato cultivars were used in this study. They were spontaneously fermented in brine solution for seven days. Titratable acidity, pH, sugar content, microbial analysis and acrylamide were analyzed on both fermented and non-fermented potatoes. Titratable acidity was the lowest in non-fermented Kirundo and Sangema at 0.01% and the highest for fermented Mabondo at 0.16%. pH wasthe highest for non-fermented Sangema pH7.30 and lowest for Fermented Mabondo at 4.31. Total viable bacteria counts ranged from 7.56log$_{10}$ CFU/g for Kirundoto 8.12 log$_{10}$ CFU/g for Sangema. Yeast and mould ranged from 4.40 log$_{10}$ CFU/g for Mabondoto 4.91log$_{10}$ CFU/g for Kirundo. Reducing sugars ranged from 0.03% for fermented Kirundo and Sangema to 0.17% for non-fermented Mabondo. Non-reducing sugars ranged from 0.07 % for fermented Kirundo to 0.80% for non-fermented CIP393251.64. Total sugars ranged from 0.09% for fermented Kirundo to 0.45% for non- fermented Mabondo. There was interaction effect on fermented and non-fermented potatoes on reducing sugars and total sugars, while interaction effect was not observed fornon-reducing sugars. Acrylamide content reduced to nearly by half for French fries and slightly more than half for crisps. Fermentation increases acidity, reduces sugars content, pH, acrylamide and contributes to to the stability and palatability of food.

Keywords:*Acrylamide, Cultivars, Fermentation, Potato, Spontaneous, Sugars*

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1. INTRODUCTION

Fermentation has been used by people in different areas of the planet since time immemorial and it has been found to increase storage stability, palatability and availability of nutrients in fermented products (Steinkraus, 2002; Ray and Sivakumar, 2009). Fermented foods are food substrates that are conquered by edible micro-organisms whose enzymes especially amylases, proteases and lipases hydrolyze the polysaccharides, proteins and lipids to non-toxic products with flavour, aromas and textures pleasant and attractive to the human consumers (Ray and Sivakumar, 2009). During fermentation there is decrease of pH which is an indicator that fermentation is taking place and it imparts the sourness to the products (Ray and Sivakumar, 2009). Fermentation of vegetables is mainly predominated by lactic acid bacteria (LAB). It was reported that LAB increased while other microbes reduced, reduction in other undesirable microbes like coliforms indicates their inhibition by LAB, this also indicates that they do not contribute in the acidification of fermented products (Kakou et al., 2010).
pH contributes to the preservation and development of aroma and flavour of fermented products (Montet et al., 2014).It lowers water activity and inhibits the growth of non-salt tolerant microorganisms comprising pathogens (Das et al., 2016). Salt together with acid inhibit growth of undesirable microorganisms and delay enzymatic softening.

It was reported that insufficient salt leads to enzyme softening of sauerkraut which leads to products of undesirable flavour and salt concentration of 2 to 3% was suggested for fermentation of cabbage (Das et al., 2016).
Fermentation increases safety and availability of food. During fermentation there is production of compounds which inhibit growth of harmful microorganisms in food. These compounds include lactic acid, alcohol, acetic acid and high salt used during fermentation (Steinkraus, 2002). These compounds also contribute to the pleasant flavour, aroma and texture of fermented food (Steinkraus, 2002;Ray and Sivakumar, 2009). At the same time fermented foods are enriched with vitamins, proteins, essential amino acids, and essential fatty acids (Steinkraus, 2002). Fermented vegetables are safe and healthy due to suppression of pathogenic microbes during fermentation and production of healthy compounds.
Sugars are the main substrate in lactic acid fermentation. Reduction of sugars was reported during fermentation of fufu from 5.21 to 4.41% (Sobowale et al., 2007). Moreover, fermentation was reported to reduce sugar content in bothfermented sweet and bitter cassava (Kakou et al., 2010). Low amount of sugars is desired in fried products. Potatoes with low reducing sugars contribute to the production of high quality fried products with low acrylamide formation. The current study aims at investigating the effect of fermentation on acrylamide formation in crisps and French fries.

2. MATERIALS AND METHODS

Potatoes were grown in Busogo farm of the University of Rwanda located in Musanze district, Northern Province of Rwanda. It is geographically located at 1°33'26'' S and 29°32'39''E. The site is characterized by Andosol due to volcanic soil. The average temperature is 16.2°C with average annual rainfall of 1420 mm (Climate-Data.Org, 2016). Factorial experiment(potato cultivars x fermentation) was conducted in three replications and five treatments representing cultivars known as factors in this experiment. They included Kinigi, Kirundo, Mabondo, Sangema and CIP 393251.64.

Sample preparation

The method used by Panda *et al.*(2007) was adopted. Peeled and washed potatoes of 140 g were submerged in a container of 500 ml containing 300 ml of 2% brine solution. For French fries, potatoes were cut into pieces of equal size of length 3-5 cm, width 1-2 cm and thickness 2-4 mm and washed for 2 minutes to remove adhering starch, while for crisps , peeled potatoes were sliced in 1.2-1.3 mm thick and washed in running tap water for 2 minutes to remove adhering starch. It was allowed to undergo fermentation at room temperature for seven days using spontaneous fermentation. Once in two days, sampling of each sample of fermented potato was analyzed for pH and total titratable acidity. After completion of fermentation, potatoes were analyzed for reducing sugars, non-reducing sugars, acrylamide, and microbial content.

Determination of pH and titratable acidity

The method of Guetouache and Guessas (2015)was used for pH and titratable acidity which were measured once in two days during seven days of fermentation. For pH measurement, 5 g of fermented potatoes were ground and dissolved into 25 ml of distilled water and pH was measured using a pH meter initially calibrated with a buffer solution at pH 4 and 7. For titratable acidity, 25 ml of sample was transferred into a beaker and 5 drops of phenolphthalein 1% indicator was added and the sample was titrated with 0.1N NaOH until the end point or pink colour was obtained.

Microbial analysis of fermented potato

The method of Aderiye and Ogunjobi (1998) was used. Amount of 5 g of fermented potatoes were mixed up in 45 ml peptone-physiological salt solution after sterilization and serial dilution up to a concentration of 10^5 using pour-plate method. Total aerobic mesophilic counts were done on plate count agar after 3 days of incubation at 30° C; lactic acid bacteria counts were done on De man Rogosa and Sharpe (MRS) agar containing 0.1% (w/v) of

natanycinafter 3-5 days of incubation at 30° C. The counts of yeasts and moulds was determined using the method of Guetouache and Guessas (2015) potato dextrose agar (PDA), acidified with 10% tartaric acid to pH 3.5 by incubating at 30° C for 3-5 days.

Reducing sugars and total sugars

The Lane and Eynon titration method using Fehling's solution was used for determination of reducing sugars (RS) and total sugars (TS) (AOAC, 2000). Ten grams of sample were diluted with 100 ml distilled water, agitated thoroughly to dissolve all suspended particles and afterward filtered with Whatman no 541in a 250 ml volumetric flask. From filtrate, 10 ml of diluted HCl was added and boiled for 5 min. The resultant solution was cooled and neutralized with 10% NaOH and made up to volume in a 250 ml volumetric flask using 3 drops of phenolphthalein as an indicator. The solution was titrated against Fehling's solution using 3 drops of methylene blue as an indicator and readings were recorded at the brick red end point and calculation used the formula below:

$$\text{Reducing sugars } \% = \frac{\mathbf{4.95\ (factor) x\ 250\ (Dilution)}\ X\ \mathbf{100}}{\mathbf{Weight\ of\ the\ sample\ x\ Titre\ x\ 1000}}$$

$$\text{Total sugars } \% = \frac{\mathbf{4.95\ (factor) x\ 250\ (Dilution) x\ 2.5\ X100}}{\mathbf{Weight\ of\ the\ sample\ x\ Titre\ x\ 1000}} \ldots$$

Non-reducing sugars were computed as the difference between total sugars and reducing sugars.

Determination of acrylamide formation in crisps and French fries

Acrylamide determination used Liquid Chromatography/Mass Spectrophotometer (LC-MS) as developed by Al-Taher (2012). Amount equivalent to 1 g of sample was measured into 50 ml centrifuge tube and 5 ml of hexane was added and vortexed. Thereafter, 10 ml of distilled water and 10 ml of acetonitrile were added followed by 0.5 g of NaCl and 4 g of $MgSO_4$ and manually shaken for one minute followed by centrifugation for 10 minutes at 2000 x g and the upper hexane layer was discarded. One ml of upper layer of acetonitrile was pipetted into a 2 ml micro centrifuge vial packed with 50 mg of Primary Second Amine (PSA) and 150 mg of $MgSO_4$and vortexed for 30 seconds. It was thereafter centrifuged for 2 minutes at 4000 x g and 500 µl was pipetted into auto-sampler vial. For standard, acrylamide stock solution (1mg/1ml) was prepared by homogenizing 100 mg of acrylamide in 100 ml of acetonitrile and kept at 4° C for further use. Preparation of internal standard (methacrylamide) stock solution (100, 000 µg/ml) consisted of pipetting 0.5 ml of the 1 mg/ml standard into 50 ml acetonitrile and kept at 4°C. Daily preparation of all working solutions was done by using acetonitrile for serial dilution. Instrument conditions were, column was reversed C-18 column (2.1 mm x 150 mm, 3 µm); column temperature of 30° C, isocratic mode (%B) of 2.5% methanol 97.5% of 0.1% formic acid, flow rate of 0.2 ml/min, injection volume of 10 µl, run time of 7 minutes and post run time of 3 minutes. Mass spectrophotometer was positive electrospray ionization mode with jet stream technology, capillary voltage of 4000 volts, nozzle voltage of 500 V, sheath gas temperature of 325° C at 5l liters/minute, drying gas temperature of 350° C at 11 liters/minutes. Acrylamide calibration curve was plotted as 0, 100, 200, 300, 400, 500, 600, 700, 800, 900, and 100 ng/ml against peak area.

Statistical analysis: Data were subjected to analysis of variance (ANOVA) and means separated by the Turkey's test at 5% level of significance using Statistical Analysis System (SAS version 9.2) with General Linear Model Procedure (Proc GLM) (SAS institute Inc., 2008).

3. RESULTS AND DISCUSSION

Effect of potato fermentation on pH

During fermentation, pH of fermented potatoes reduced from 7.30 for Sangema to 4.31 for Mabondo as depicted in Figure 1.

The effect of fermentation with time was statistically significant at ($P < 0.05$). After adding brine solution to potatoes, a slight increase in pH was observed which may be related to the alkaline nature of salt used. Decrease in pH was faster in potato with high sugar content that in potato with low sugar content. During lactic acid fermentation there is reduction of pH and increase of acidity which altogether help to preserve food. pH of fermented sweet potatoes with *Lactobacillus plantarum* was reported to reduce from 5.5 to 2.6 after seven days of fermentation and after 28 days pH remained constant (Panda *et al.*, 2007). Similarly, fermentation of boiled and non-boiled sweet potatoes with *L. plantarum* showed reduction in pH from 6.1 to 3.3 for boiled and from 5.8 to 2.2 for non-boiled sweet potatoes (Panda and Ray, 2007).

Fermentation of potatoes with *L. plantarum* of 10^9 CFU/ml reduced pH from 5.70 to 4.05 after 3 hours of fermentation (Baardseth *et al.*, 2006). Reducing pH to 4 or less increases stability and safety of products due to suppression of harmful bacteria (Montet *et al.*, 2014). Lowering pH by lactic acid or acetic acid had bactericidal and bacteriostatic effect, and in addition there is production of H_2O_2 and bacteriocins which also play a role of microbial inhibition (Agrawal, 2005). Fermentation improves food security, nutrition quality, aroma, flavour, texture and removes anti-nutrients from fermented vegetables (Demir *et al.*, 2006). pH is responsible for the development of aroma and flavour of fermented fruits and vegetables (McFeeters, 2004). Low pH helps to stabilize food due to microbial growth inhibition and contributes to the flavour and aroma of fermented products.

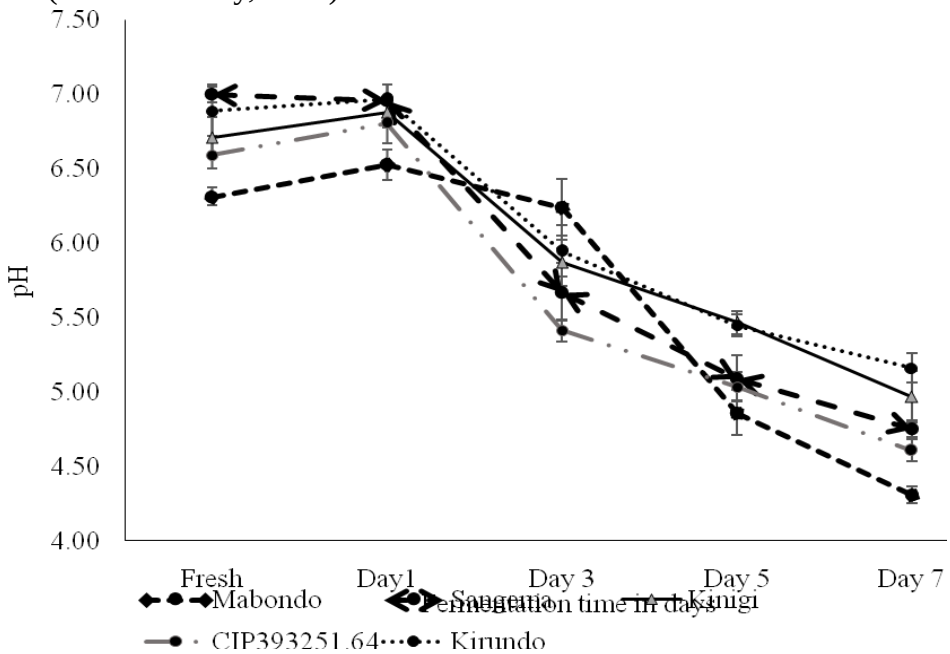

Figure 1. pH of potatoes during fermentation period
Error bars represent standard error of the mea

Changes in titratable acidity of fermented potatoes

Titratable acidity increased during fermentation time. There was a significant increase of titratable acidity with fermentation time of potatoes at ($P < 0.05$). Initial titratable acidity of potatoes in this study ranged from 0.01 for Kirundo and Sangema to 0.02% for Kinigi, Mabondo and CIP393251.64 and after seven days of fermentation it was 0.10 for Kirundo to 0.16% for Mabondo as shown in Figure 2.

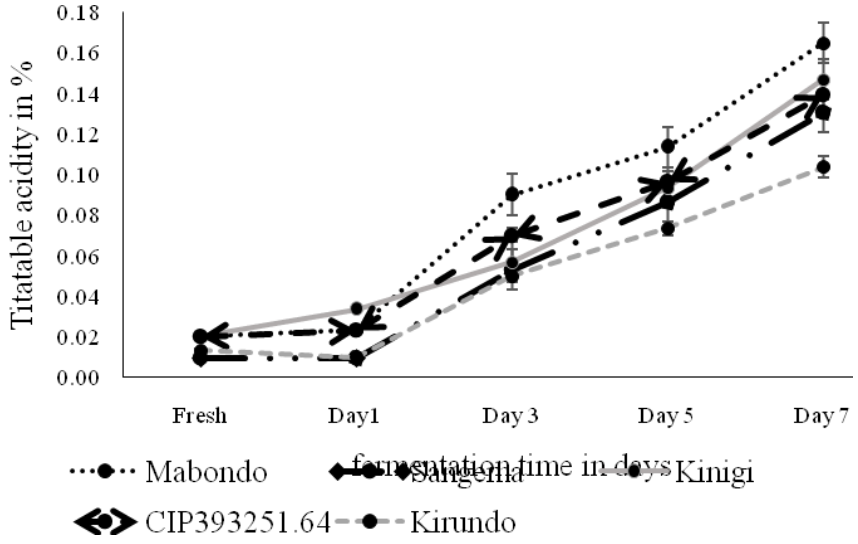

Figure 1. Titratable acidity of potatoes during fermentation period
Error bars represent standard error of the means

Acidity increased gradually during fermentation period. Increase was faster in potatoes with high sugar content than in potatoes with low sugar content. Similarly, titratable acidity during seven days of roots fermentation was reported to be 0.08% before fermentation and increased in the range of 0.23-0.66% and the increase in titratable acidity was inversely proportional to the concentration of brine solution and the titratable acidity remained the same after 28 days of fermentation (Panda *et al.*, 2007). Titratable acidity increased from 0. 45% to 0.6% and 2 % for natural and controlled sauerkraut in 15 days of fermentation (Pandey and Garg, 2015). Fermentation of boiled and non-boiled sweet potatoes with *L. plantarum* showed titratable acidity from 0.08 to 0.123% for boiled and from 0.07 to 0.146% non-boiled sweet potato (Panda and Ray, 2007). Lactic acid fermentation increases acidity by converting sugars into acid where the main byproduct is lactic acid. High acidity contributes to the stability of fermented products due to its ability to prevent harmful microbial growth.

Microbial population of fermented potato

During fermentation microbial population was evaluated after fermentation time. Total viable bacteria count was the highest which ranged from 7.56 $\log_{10}$ CFU/g for Kirundo to8.12 $\log_{10}$ CFU/g for Sangema. The total count represents all types of living microorganisms in fermented potatoes. The number of total microbial count in different potato cultivars was statistically significantly different at ($P < 0.05$). It was likely to be high where sugars were high and sugars might have facilitated their quick development. Yeast and mould ranged from 4.40 $\log_{10}$ CFU/g for Mabondoto 4.91 $\log_{10}$ CFU/g for Kirundo. Yeast and mould also participate in fermentation. Lactic acid bacteria ranged from 6.43 $\log_{10}$ CFU/g for Kinigito 7.39 $\log_{10}$ CFU/g for Sangema as shown in Table 1.

Table 1. Microbial population of fermented potato

Cultivars	Total viable count log_{10}	Yeast and mould log_{10}	LAB log_{10}
Mabondo	8.12 ± 0.06^{a}	4.91 ± 0.00^{a}	7.13 ± 0.05^{b}
Sangema	8.22 ± 0.04^{a}	4.53 ± 0.10^{b}	6.22 ± 0.03^{c}
Kinigi	7.56 ± 11^{b}	4.55 ± 0.01^{b}	7.39 ± 0.02^{a}
CIP393251.64	7.57 ± 0.03^{b}	4.56 ± 0.03^{b}	6.50 ± 0.02^{c}
Kirundo	7.56 ± 0.01^{b}	4.40 ± 0.03^{b}	6.43 ± 0.02^{c}
Minimum	7.56	4.40	6.22
Maximum	8.22	4.91	7.39
CV	1.12	1.73	0.63
MSD	0.2466	0.2234	0.119

MSD: Minimum significant difference; Means followed by the same letter in a column do not differ by Tukey's test at 5%.

The number of lactic acid bacteria, yeast and mould in all cultivars was statistically significantly different at ($P < 0.05$). Fermentation of yam showed reduction in total viable microbial count from 6.02 for the first day to 5.75 log_{10} CFU/ml for the fifth day, lactic acid bacteria increased from 1.90 for the first day to 3.90 log_{10} CFU/ml at the fifth day (Aderiye and Ogunjobi, 1998). Growth of lactic acid bacteria is responsible for production of lactic acid and acetic acid which reduced pH and impended growth of Gram-negative and sporulating bacteria (Montet *et al*., 2014). Fermentation is initiated by *Lactobacillus mesenteroides* which is heterofermentative able to utilize glucose and fructose in fermentation which quickly lowers pH that inhibits growth of undesirable microorganisms and activity of their enzymes (Pandey and Garg, 2015). Along with lactic and acetic acids, hydrogen peroxide and carbon dioxide are produced and they also have bactericidal effect (Montet *et al*., 2014). Carbon dioxide produced inhibits growth of aerobes like *Bacillus, Pseudomonas* and *Micrococcus* (Pandey and Garg, 2015). Lactic acid bacteria help to preserve food by producing lactic acid and other byproducts which help to suppress growth of harmful microorganisms.

Sugar changes during fermented potato

During fermentation there was change in sugars content. The effect of cultivars and fermentation was statistically significant at ($P < 0.05$) except for non-reducing sugars as presented in Table 2.

Table 2. Interaction effect among potato cultivars and fermentation on sugar content for 100g of fresh weight

Cultivars	Treatments	Reducing sugars %	Non-reducing sugars %	Total sugars %
Kinigi	Non-Fermented	0.13 ± 0.01^{b}	0.21 ± 0.02^{a}	0.33 ± 0.03^{b}
	Fermented	0.05 ± 0.00^{de}	0.10 ± 0.01^{a}	0.14 ± 0.01^{def}
Kirundo	Non-Fermented	0.08 ± 0.01^{cd}	0.14 ± 0.01^{a}	0.23 ± 0.02^{cd}
	Fermented	0.03 ± 0.01^{e}	0.07 ± 0.01^{a}	0.09 ± 0.01f
Mabondo	Non-Fermented	0.17 ± 0.02^{a}	0.28 ± 0.04^{a}	0.45 ± 0.06^{a}
	Fermented	0.06 ± 0.01^{cde}	0.13 ± 0.02^{a}	0.19 ± 0.3^{de}
Sagema	Non-Fermented	0.09 ± 0.00^{c}	0.19 ± 0.01^{a}	0.29 ± 0.01^{bc}
	Fermented	0.03 ± 0.00^{e}	0.80 ± 0.00^{a}	0.12 ± 0.01^{f}
CIP393251.64	Non-Fermented	0.13 ± 0.01^{b}	0.22 ± 0.01^{a}	0.35 ± 0.02^{b}
	Fermented	0.05 ± 0.01^{de}	0.10 ± 0.00^{a}	0.15 ± 0.01^{def}
CV		14.55	16.35	12.81
MSD		0.0354	0.0721	0.0882

MSD: Minimum significant difference at 5% Tukey; Means followed by the same letter in a column do not differ by Tukey's test at 5%

Sugar content reduced during fermentation period. There was interaction effect between cultivars and fermentation on reducing sugars at ($P < 0.05$) with reducing sugars varying from 0.03% for fermented Kirundo and fermented Sangema to 0.17%for non-fermented Mabondo. There was no interaction effect between cultivars and fermentation on non-reducing sugar ($P > 0.05$) varying from 0.07% for fermented Kirundo to 0.28% for non-fermented Mabondo. The interaction effect was statistically significant between cultivars and fermentation at ($P < 0.05$) ranging from 0.09% for fermented Kirundo to 0.45% for non-fermented Mabondo. There was reduction in all types of sugars during fermentation.

Fermentation of boiled and non-boiled sweet potatoes with *L. plantarum* showed reduction in total sugars from 2.10 to 1.12% for boiled and 2.14 to 1.19% for non-boiled, reducing sugars reduced from 0.75 to 0.35% for boiled and 0.80 to 0.42% for non-boiled sweet potatoes (Panda and Ray, 2007). It was further reported that during fermentation total sugars reduced from 0.66% to 0.12% after 28 days of fermentation (Panda *et al.*, 2007). Likewise, for fermented potato with *L. plantarum*, glucose reduced from 0.61% to 0.029%, fructose reduced from 0.46% to 0.00%, sucrose reduced from 0.13 to 0.029% (Baardseth *et al.*, 2006). Reduction of sugars during fermentation is related to their utilization as source of energy for fermenting bacteria. They are fermented by sugars into lactic acid and other byproducts. Potato fermentation is important for reduction of sugars due to their contribution on quality of products which is objectionable if high.

Acrylamide formation during frying of fermented and non-fermented French fries and crisps

Results of acrylamide content in fermented and non-fermented crisps and French fries are presented in Figure 3. Acrylamide is among byproducts of Maillard reaction due to the reaction between asparagine and reducing sugars. The difference in acrylamide of potato tubers analyzed was statistically significant at ($P < 0.05$). Acrylamide of French fries ranged from 376.52µg/kg for Kinigi to 629.63µg/kg for CIP393251.64 and after fermentation it ranged from 267.73µg/kg for Kirundo to 306.00µg/kg for Mabondo. For crisps, acrylamide ranged from 767.00µg/kg for Kirundo to 855.30µg/kg for Mabondo.

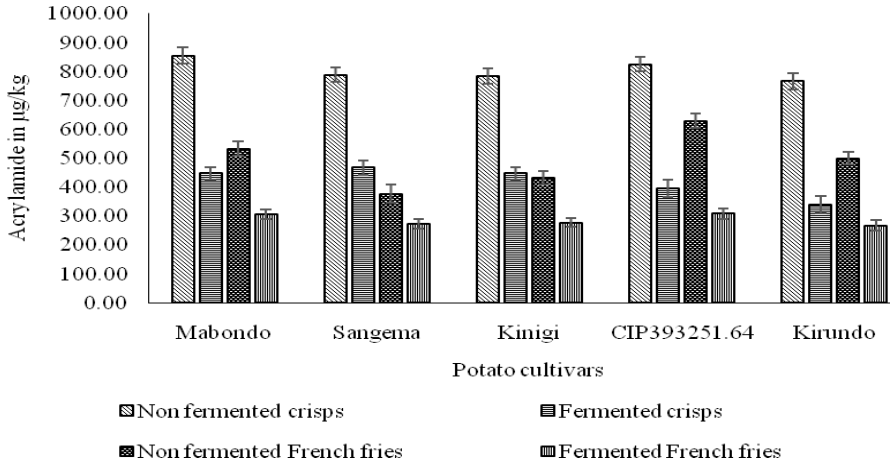

Figure 2. Level of acrylamide in fermented and non-fermented French fries and crisps in µg/kg of wet weight.
Error bars indicate standard error of means

Fermented crisps had 339.59µg/kg for Kirundo to 468.05µg/kg for Sangema. Acrylamide in fried French fries and crisps was reported to range from 30 to 2300 µg/kg, where it is around 424 µg/kg for French fries and 1739 µg/kg for crisps (Singh and Kaur, 2009) which aligns with the results of this study. Ordinary, the range of acrylamide in French

fries is 300 to 700 µg/kg with extreme of 300 to 3500 µg/kg, while for crisps is from 600 to 2000 µg/kg with extreme values of 170 to 2300 µg/kg (Lingnert *et al.*, 2002). Acrylamide formation can be reduced when factors which influence its formation are taken into consideration during frying.

Acrylamide formation is influenced by factors like agronomic factors, recipe factors and processing factors (Stojanovska and Tomovska, 2015). Agronomic factors include cultivars genetic makeup like the amount of glucose, fructose and free asparagine, harvesting time, climatic conditions, soil composition and agronomic practices (Stojanovska and Tomovska, 2015). Sucrose can be hydrolyzed to give glucose and fructose which are precursor of acrylamide, while lowering pH using organic acid reduced acrylamide formation, acrylamide also reduces with pretreatment like soaking, and blanching (Stojanovska and Tomovska, 2015). Fructose accelerates acrylamide formation than glucose where at 140^{o} C fructose favoursacrylamide formation, while glucose required temperature above 140^{o} C and it was found that acrylamide formation starts as soon as sugar starts to melt, melting temperature for fructose was found to be 127^{o} C and 150^{o} C for glucose (Ciesarová *et al.*, 2006). Reducing sugars like fructose and glucose were reported to positively correlate with acrylamide and negative correlation for sucrose a non-reducing sugars (Zhu *et al.*, 2010). Asparagine was reported to correlate positively with acrylamide, while other amino acids did not or correlated poorly with acrylamide and total amino acids did not also correlate with acrylamide (Zhu *et al.*, 2010). Fermentation helps to reduce sugar content in return contribute in reduction of acrylamide formation during frying.

4.CONCLUSION

Fermentation of vegetables is related to the transformation of sugars by lactic acid bacteria into lactic acid and other byproducts. During fermentation there is increase in lactic acid bacteria which results in reduction of pH and increase in acidity. Moreover, sugars are fermented which leads to reduction of reducing sugars which improves quality of fried products and less acrylamide production.

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