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Research Article

Acrylamide Formation during Baking of Whole Wheat Flour Incorporated with Spent Coffee Grounds and Juices of Lemon Fruits and Rosemary Leaves

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Whole wheat bread is increasingly consumed worldwide. The objective of this study was to evaluate the effect of the addition of spent coffee grounds (SCG), juice of lemon fruits (L) and juice of rosemary leaves (R), and fermentation duration on the acrylamide reduction in whole wheat breads. The dough, comprising whole wheat flour (W), SCG, L, R, and salt, was fermented for 60 min and for 120 min, separately, and baked at 180°C for 20 min. Data were subjected to one-way analysis of variance (ANOVA) using SAS System for Windows. Tukey's test was used to differentiate between treatment means. The least significant difference was accepted at $p \leq 0.05$. Control (with no SCG, L, or R added) whole bread from the Gihundo variety had the highest content of acrylamide (47.23 μg kg^{-1}). Dough incorporated with ingredients (Gihundo W: SCG+LR) gave bread (Gihundo W:SCG+LR) with the lowest acrylamide content (10.5 μg kg^{-1}). The acrylamide content was significantly higher in all breads from dough fermented for 60 min than that of the breads from dough fermented for 120 min. The results of the current study support the use of SCG+LR and an extended fermentation duration as techniques to reduce the amount of acrylamide in whole wheat breads.

1. Introduction

Studies were conducted and published on the health benefits of consuming whole-grain wheat products [1]. This has led to the growing availability of whole wheat bread on the marketplace [2]. This type of bread is a rich source of strong health-promoting compounds such as dietary fiber, mainly concentrated in the outer portions (bran) and germ of the grain [3]. However, the researchers have found more acrylamide content in whole wheat bread than that found in refined or wheat bran breads [4]. Acrylamide is a chemical compound formed during the Maillard reaction and was reported to cause possible carcinogenicity, neurotoxicity, and reproductive and developmental toxicity effects to consumers. The Maillard reaction occurs between amino compounds (principally amino acids) and carbohydrates during heating (baking, frying, roasting, and grilling) above 100°C [5, 6]. The level of acrylamide formation is favored by the heating temperature, time, type and concentrations of sugars, amino acids, pH, water activity, leavening agents, and antioxidants [7]. Acrylamide formation in food is the glucoconjugate of the asparagine with the presence of reducing sugar or a compound with carbonyl group at high temperature and low moisture [8]. Specifically, asparagine and reducing sugars are the limiting precursors for acrylamide formation in cereal and potato products, respectively [9, 10]. So far, a number of mitigation strategies to reduce the acrylamide content in foods have been proposed and tested at laboratory and pilot plant scales. This includes the selection of foods such as raw material low in acrylamide

precursors, processing temperature/time, and addition of some amino acids like glycine, cysteine, organic acids and acidulants, calcium ions, cyclodextrin, and natural antioxidants or antioxidant extracts. Sazesh and Goli [11] reported a significant reduction of the acrylamide content in biscuit when they optimized formulation by wheat flour substitution with quinoa flour at five levels (0%, 25%, 50%, 75%, and 100% based on wheat flour in formulation), using different levels of sodium bicarbonate at three levels (0.05%, 0.1%, and 0.15% based on the final dough weight) and baking temperature at three levels (160°C, 185°C, and 210°C). Quinoa flour was used because it contains low levels of fructose and glucose and much lower amount of asparagine in relation to wheat flour as more effective precursors participating in the Maillard reaction. The use of asparaginase and the replacement of reducing sugars with sucrose are also listed among reduction strategies [12]. The growing awareness about health issues arising from food consumption preserved with chemicals has led to improving food safety by using natural preservatives. Spent coffee grounds (SCG) as food waste, juice of lemon fruit, and juice of rosemary leaves were used as an enrichment in bread formulation. The antioxidants found in SCG were assumed to lower the Maillard reaction for the acrylamide formation in biscuits [13–15]. Hedegaard et al. [16]; Zhang and Zhang [17]; Zhang et al. [18] showed that there was in substantial reduction in acrylamide levels in bread depending on the nature of the antioxidants from the natural extract. These phytochemicals can mitigate acrylamide formation by free radical scavenging activity or by other ways including trapping carbonyl moiety or/and precipitating asparagine and components such as proteins, peptides, saccharides, and monovalent/divalent cations [19–21]. The purpose of this study was to evaluate the effect of the addition of spent coffee grounds (SCG), juice of lemon fruits (L) and juice of rosemary leaves (R), and fermentation duration on the minimization of acrylamide contents in whole breads from four (4) wheat grain varieties. The doughs comprising whole wheat flour (W), SCG, L, R, and salt were fermented for 60 min and for 120 min, separately, and baked at 180°C for 20 min. The contents of free asparagine, fructose, and glucose; the antioxidant activity; and pH were analyzed as some of the factors that can influence the formation of acrylamide in whole wheat bread.

2. Materials and Methods

2.1. Collection of the Samples. Four (4) dry wheat grain varieties, namely, TAI, EN161, Eagle10, and Korongo with the local names Gihundo, Kibatsi, Nyaruka, and Reberaho, respectively, were collected from the stores of Rwanda Agriculture and Animal Resources Development Board (RAB), located at Kinigi, Musanze district, Rwanda. These wheat varieties were grown in RAB farms at the temperature varying between 16.6 and 21.5°C, at the same location, and under the same agroecological and cultural conditions in the crop year 2018. The wheats were grown in volcanic soil and fertilized with urea and diammonium phosphate (DAP). The wheat grains were sampled in the same year, packed in high-density polyethylene bags, and stored at room temper-

ature prior to milling. The wastes, known as spent coffee grounds, were directly taken after brewing coffee (Coffea arabica var. bourbon) in a coffee shop in Musanze town, Rwanda, and stored in a transparent plastic bottle in a freezer (SM302NW, SM302NW1014009, 2010/08, Shandong, China) at -20°C prior to analysis. Fresh green lemon fruits (var. African rough lemon) and raw green rosemary leaves (var. Arp rosemary herb) were bought from the market in Musanze town, Rwanda, packed in plastic sachets, and stored at 5°C in the refrigerator (Hisense, HBM17158SS, 2015, Shandong, China) prior to processing. The coffee, lemon, and rosemary were all grown in Rwanda.

2.2. Preparation of the Raw Samples

2.2.1. Wheat Grain Milling. Wheat grains were collected from the stores of Rwanda Agriculture and Animal Resources Development Board (RAB), located at Kinigi, Musanze district, Rwanda, in the crop year, September 2018. Before milling, the wheat grains were conditioned to 15.5% moisture content in order to get a fine particle size whole wheat flour by the addition of distilled water and were left for at least 24 hr at ambient conditions in a closed plastic container for the absorption of the moisture [22]. The whole wheat grains were ground by passing through a model 3100 hammer mill (Falling Number AB, Huddinge, Sweden) equipped with 1 mm screen. AACC Approved Method 26-95.01 [23] was used to calculate the amount of water to be added for wheat grain tempering.

$$\text{ml} = \left(\left(\frac{100\text{-\%moisture}}{100\text{-}15.5\%} \right) - 1 \right) \times \text{grams of wheat grains.} \quad (1)$$

The conditioned wheat grains of each variety were wholly milled by using a laboratory hammer mill (CM 1090 Cemotec, 2009, Henan, China). All bran and germ were mixed with the flour. The flour was packed in high-density polyethylene envelop and stored at -20°C in a freezer (SM302NW, SM302NW1014009, 2010/08, Shandong, China) prior to analysis and baking.

2.2.2. Lemon Fruit and Rosemary Leaf Juices Making. The lemon (var. African rough lemon) and rosemary (var. Arp rosemary herb) juices were obtained by using a blender (Moulinex, LM241, Genuine, 2017, Paris, France). The lemon fruits (3 kg) were washed and peeled with a knife. The rosemary leaves were picked off the woody branch by using hand and washed. Then after, they were cut into quarters and juiced by using a blender (Moulinex, LM241, Genuine, 2017, Paris, France) with no water added for between 30 secs and 1 min until the extraction is complete. Rosemary leaves (5 kg) were ground in the blender (Moulinex, LM241, Genuine, 2017, Paris, France) between 2 and 4 min until all leaves were thoroughly ground with no water added. To get the clear juice, lemon fruit pulp and ground rosemary leaves were separately squeezed in a cheesecloth (grade 90, 44 × 36 weaves; 2018, Zhuojie, China) by using hand. The wastes (peels, seeds, and other solid materials resulting from the extraction of juices were deposited in the appropriate

bin. The clarified lemon and rosemary juices were kept in transparent plastic bottles and stored at 6°C in a fridge (SM302NW, SM302NW1014009, 2010/08. Shandong, China) prior to analysis and processing.

2.3. Freeze Drying of Dough.

Dough was put in a freezer (SM302NW, SM302NW1014009, 2010/08, Shandong, China) at -20°C. After that, it was placed in a freeze dryer (Christ, Alpha 2-4 LS plus, SN: 24/61/11/2017, Darmstadt, Germany) for 24 hours at -82°C and 0.1 mbar vacuum to finish drying. For further analysis, dried dough was crushed into fine flour using a laboratory grinder (Moulinex, LM241, Genuine, 2017, Paris, France).

2.4. Baking.

The dough comprised 200 g whole wheat flour from each of the wheat variety grains (TAI, EN161, Eagle10, and Korongo), 2% instant dry yeast (GB Ingredients, Dordrecht, 2019, Holland), 2% sodium chloride, and potable water (125 mL) [24]. The amounts of 4% spent coffee grounds [14], 1% lemon fruit juice, and 1% rosemary leaf juice were added. The electric balance (Explorer EX 223, version 2.00/2.00. SN B 333687045, IR Sensor, OHAUS, NJ, USA) was used. After being mixed in a dough mixer (Combisteel Dough Mixer Liter, 7455.1400, 2011, China), the mixture was fermented in a fermenter (Manz Backtechnik GmbH, Creglingen, Germany) at 34°C, 60% relative humidity for 60 minutes and at 39°C, 85% relative humidity for 120 minutes [25]. Rotation of the mixer was 54 rotations per minute (rpm) for 3 min until the dough came together and then switched to 104 rpm for 6 min. Each fermented dough was covered with a lid and baked at 180°C for 20 min in an oven (electric baking oven, Lemarkz, model: LGO-24A, 2010, Mumbai, India).

The control bread from each wheat variety was made for 60 min of fermentation without incorporation of SCG and LR. The loaves were depanned, cooled for two hours, and then sealed in unperforated low-density polythene bags and placed in a freezer (SM302NW, SM302NW1014009, 2010/08, Shandong, China) where they were kept at -20°C until analysis.

2.5. Determination of Chemical Parameters

2.5.1. Free Asparagine Content for Whole Wheat Flour, Dough, Bread, Spent Coffee Grounds, and Lemon Fruit and Rosemary Leaf Juices.

The determination of free asparagine (asn) was done by following the method of Hamlet et al. [26]. Stock standard solutions of all amino acids were prepared at 1 mg ml^{-1} in distilled water. The preparation of mixed standards was done by blending and serially diluting the stock standards with distilled water to give calibration standards. To prepare the reagent for the derivatization, ten (10) mg of o-phthaldialdehyde (OPA) was dissolved in 100 μl of methanol, making 1 ml with borate buffer (0.4 M and pH 10.2). 20 μl of 3-mercaptopropionic acid (3-MPA) was then added. The derivatization procedure was the same for standard and samples. Analysis of derivatized asparagine was performed on a UPLC (LCMS-8050) linked with a fluorescence RF-535 detector (Shimadzu, Kyoto, Japan) using a LichroCart Lichrosphere RP-18 column (250 mm × 4 mm with 5 μm diameter particles) (Merck, Darmstadt, Germany). The HPLC mobile phase was prepared using 825 ml of 50 mM potassium phosphate buffer (adjusted to pH 7 with 50% (v/v) sodium hydroxide) plus 145 ml acetonitrile and 30 ml tetrahydrofuran. 2.5 g of the extracted sample using 20 mL of acetic acid was weighed and incubated. The supernatants were adjusted to 100 ml with 0.01 M acetic acid. Aliquot of 3 ml of the sample, 50 μl of borate buffer, and 50 μl of OPA reagent were added into the sample derivatization vial containing 800 μl water. The mixture of an aliquot (3 ml) HCl (0.01 M, 1 : 1 v/v) and 4 ml of the solution was prepared. Pipetting of HCl and solution was done with a pipette inserted with graduate cylinder (Eppendorf easypet 3) and pipette filler (SN: 181248, Thermoscientific, China). For lemon fruit and rosemary leaf juices, juices (39 ml) were diluted with 40 ml of distilled water. All solutions were shaken (Controlled Environment Incubator shaker, New Bruswik Scientific Co., Inc., Edison, NJ, USA) at 350 rpm for 30 min at 20°C and then centrifuged by using a centrifuge (5415D, Eppendorf) at 3000 rpm for 10 min. An external standard method was used to measure asparagine, and external calibration curves were created for asparagine in the range of 0.05 to 2.0 mg/L. By adding 0.5 mg/L of theanine as an internal standard to both working standards and extracts, the accuracy of the asparagine analysis was examined. Asn was expressed as mg100g^{-1}.

2.5.2. Reducing Sugars for Whole Wheat Flour, Dough, Bread, Spent Coffee Grounds, and Lemon Fruit and Rosemary Leaf Juices.

The individual sugars were determined by using the procedure for samples containing low fat and low protein [27]. For whole wheat flour, dough, bread, and spent coffee grounds, CaCO$_3$ (1 g) was added into the sample (1 g) to neutralize it. The ethanol of 25 mL 85% was also added into the sample and shaken by using water bath at 60°C for 1 h. The sample was removed from the water bath and immediately filtered through a filter paper. The extraction with 25 mL boiling 85% ethanol was done three times. The ethanol was evaporated on a rotary evaporator at 45°C until the remaining aqueous solution was approximately 3 ml. The solution was mixed with a distilled water volumetric flask up to a 10 ml mark. For lemon fruit and rosemary leaf juices, each juice (100 μl) was diluted with distilled water (1000 μl) to be vortexed by using a mixer (model no: SI_0166, SN: 16-1114, Genie Touch Mixer Scientific Industries Inc., NJ, USA) and filtered with syringe filter (hydrophilic Germany). By using a pipette (Petman, Gilson), the solution (1.5 ml) from whole wheat flour, dough, bread, spent coffee grounds, and lemon fruit and rosemary leaf juices samples was filtered through a 0.2 μm nylon filter into a HPLC vial. Each sample was analyzed by using HPLC system consisting of a GP40 gradient pump and ED40 electrochemical detector coupled with an amperometric cell and AS3500 auto sampler (Dionex Corporation, Sunnyvale, CA, USA). The HPLC column was a 250 × 4 mm 5 μm CarboPacTM PA-1, and the mobile phase was 200 mM sodium hydroxide solution (isocratic elution, 15 min).

Calculation,

$$\text{Total amount of each sugar (g/100 g)} = \frac{\text{Aspl} \times C \times \text{DF} \times 100}{\text{Astd} \times W \times 1000},$$

(2)

where Aspl is the area/peak height of each sugar in the sample solution. Astd is the area/peak height of sugar standard. C is the concentration of sugar standard (μg/ml). DF is the total dilution factor. W is the weight of the sample in grams. 100 is the conversion factor to report results in mg/100 g. 1000 is the conversion from mg/ml to g/ml.

2.5.3. Total Antioxidant Activity for Whole Wheat Flour, Dough, Bread, Spent Coffee Grounds, and Lemon Fruit and Rosemary Leaf Juices.

The total antioxidant activity was determined using DPPH procedure [28]. Two grams of whole wheat flour, dough, bread, and spent coffee grounds and 200 μl of juices were mixed with 10 ml 80% methanol, separately. The mixture was shaken by using mechanical shaker (Controlled Environment Incubator Shaker, New Brunswick Scientific Co., Inc., Edison, NJ, USA) at 25°C for 24 hr. Afterwards, the mixture was then centrifuged at 4,000 rpm for 10 min. The supernatant aliquot was taken out for determination. A working DPPH solution was prepared by diluting 200 ml of stock solution with approximately 800 ml of 50% ethanol. DPPH (50 μl) was added into supernatant aliquot sample (50 μl and was shaken by using a plate shaker (Insel Shaker, SN: 95/S89/73, Hamble SO3 8DH, Stevenage, England). The absorbance was read at 515 nm in a microtiter plate spectrophotometer reader (BioTek Instruments, SN: 216574, NJ, USA). The results were expressed as mg of Trolox Equivalents per 100 g sample.

2.5.4. Acrylamide Contents for Whole Wheat Breads and Spent Coffee Grounds.

Acrylamide content was determined by using the protocol of Al-Taher [29]. All reagents, namely, acetonitrile, n-hexane, methanol formic acid, and water, were LC/MS grade. The acrylamide standard stock solution (1 mgml^{-1}) was prepared by dissolving 100 mg of the acrylamide in 100 ml acetonitrile and stored at 4°C. The internal standard (methacrylamide) stock solution (100.000μgml^{-1}) was made up by pipetting 0.5 ml of the 1 mg/ml standard into 50 ml acetonitrile and stored at 4°C. All working solutions were prepared daily by serial dilution in acetonitrile. One gram from bread and spent coffee grounds samples was weighed and added in a 50 ml centrifuge tube from the agilent bond elut QuEChERS extraction kit. The internal standard (13C3 acrylamide) at 500 ng/g and hexane (5 ml) were also added in the tube, and then the mixture was vortexed. Water (10 ml) and acetonitrile (10 ml) were added followed by the agilent bond elut QuEChERS extraction salt mixture for acrylamides (p/n 5982-5850). The sample tubes were shaken for 1 min vigorously and centrifuged at 5000 rpm for 5 min. Separation of the chemicals was performed on a reversed-phase C18 column (2.1 mm × 150 mm, 3 μm). A gradient program was set up using 0.02 M phosphate buffer at pH 7.80 (solvent A) and 45 : 45 : 10 (v/v) acetonitrile:methanol:water (solvent B) at 1.5 mL min-1: 57% solvent B increased to 100% over 1.8 min, held for 18 min, and then decreased to 0% over 5 min. The acrylamide in the sample was determined by using HPLC/LCMS-8050 (Model: RF-20AXS, SN: L20505000663 AE, Shimadzu Corporation, Tokyo, Japan). The acrylamide content was expressed in μg kg^{-1}.

2.6. Statistical Analysis.

Data were subjected to one-way analysis of variance (ANOVA) using SAS System for Windows (version 9.3, SAS Institute, Cary, NC). Treatment means were separated using Tukey's test, and the least significant difference was accepted at $p \leq 0.05$.

3. Results and Discussion

Whole breads from 4 wheat varieties (Gihundo, Kibatsi, Nyaruka, and Reberaho) were significantly different ($p \leq 0.05$) in acrylamide contents (Table 1). The findings showed that free asparagine levels in wheat varieties, rather than their fructose and glucose contents, were directly correlated with acrylamide amounts in the resultant whole wheat breads. This was demonstrated by the fact that whole bread from the Gihundo wheat variety, which had high levels of acrylamide (47.23 μg kg^{-1}), also had high levels of free asparagine (0.399 mg 100 g^{-1}), whereas bread from Nyaruka wheat variety, which had low content of acrylamide (30.30 μg kg^{-1}), also had low amount of free asparagine (0.331 mg 100 g^{-1}). Contrarily, the quantity of fructose (0.016–0.022%) and glucose (0.024–0.037%) observed in whole wheat flours increased in all breads (0.04–0.330% for fructose and 0.380-0.80% for glucose), rather than decreasing. The similar results were reported in the work done by Rwubatse [30]. This suggests that fresh fructose and glucose were formed during the baking of wheat flour in the whole loaf, as reported by Westerlund et al. [31]. Amrein et al. [9]; Stadler [32]; Claus et al. [33] found the same by discovering that more than 99% of the acrylamide formed in the bread crust and that the bread crust had high levels of reducing sugars (0.40-0.64 g of fructose and 0.25-0.35 g of glucose per 100 g of dry crust). It showed that the main limiting precursor for acrylamide synthesis in yeast-leavened whole breads was free asparagine, not fructose or glucose. The current findings are consistent with those of Blank et al. [8], who noted that the glycoconjugates of asparagine are the main source of acrylamide in foods when reducing sugars or an appropriate carbonyl source is present under high temperatures.

Therefore, it is crucial to carry out particular treatments on wheat cultivars to combat asparagine development. Researchers in the UK have reported genetic modification of wheat to prevent the accumulation of asparagine by altering the expression of asparaginase [34]. Wheat sprouting results in an increase in protease activity and nitrogen transport, which results in a high asparagine content.

Addition of SCG+LR in doughs reduced significantly ($p < 0.05$) the acrylamide formation in control (with no added SCG or SCG+LR) breads from 47.00 μg kg^{-1} to 10.5 μg kg^{-1} (Table 2). This was due to free asparagine which was not detected in these ingredients (Table 2). The acrylamide contents of the breads were not increased by the incorporation of SCG as this ingredient showed low concentration of acrylamide (5.41 μg kg^{-1}) with regard to those of control breads. In another study carried out by Sazesh and Goli [11], the decrease in acrylamide levels was noticed when quinoa flour was used to make biscuits. The authors

TABLE 1: Variation in acrylamide content in whole breads from different wheat varieties.

Wheat variety	Asparagine content (mg 100 g^{-1})	Fructose content (%)	Glucose content (%)	Asparagine content (mg 100 g^{-1})	Fructose content (%)	Glucose content (%)	Acrylamide content (μg kg^{-1})
		Whole flours			Whole breads		
Gihundo	0.399 ± 0.06^a	0.022 ± 0.01^a	0.037 ± 0.07^a	0.050 ± 0.001^a	0.380 ± 0.04^a	0.330 ± 0.06^a	47.23 ± 0.92^a
Kibatsi	0.372 ± 0.02^b	0.020 ± 0.02^b	0.03 ± 0.01^b	0.051 ± 0.002^a	0.330 ± 0.02^b	0.260 ± 0.02^b	31.10 ± 1.41^b
Nyaruka	0.331 ± 0.04^d	0.016 ± 0.03^d	0.024 ± 0.04^c	0.049 ± 0.002^{ba}	0.280 ± 0.08^d	0.210 ± 0.06^d	30.30 ± 0.82^d
Reberaho	0.361 ± 0.09^c	0.017 ± 0.01^c	0.03 ± 0.02^b	0.046 ± 0.002^c	0.300 ± 0.01^c	0.230 ± 0.01^c	30.50 ± 0.98^c

Values are means ± SD of 3 replications. Means with different superscripts within a column are significantly different at $p \leq 0.05$. Dough was fermented for 60 min.

TABLE 2: Effect of spent coffee grounds, juice of lemon fruit, and juice of rosemary leaves on acrylamide contents in whole wheat breads.

Wheat variety	Whole flours +ingredients	Antioxidant capacity (mg 100 g^{-1})	pH	Antioxidant capacity (mg 100 g^{-1})	pH	Acrylamide content (μg kg^{-1})
			Whole doughs		Whole breads	
	SCG	25.84 ± 0.23^b	6.14 ± 0.38^b			5.41^m
	L	8.08 ± 0.11^j	2.7 ± 0.24^m			
	R	26.77 ± 0.25^a	5.82 ± 0.17^e			
Gihundo	W	10.88 ± 0.18^n	6.22 ± 0.89^a	7.48 ± 0.76^k	5.81 ± 0.37^a	47.23 ± 0.92^a
	W:SCG	22.12 ± 0.21^d	5.82 ± 0.23e	11.6 ± 0.78^e	5.80 ± 0.35^b	16.90 ± 0.80^k
	W:SCG + LR	23.1 ± 0.37^c	5.35 ± 0.32^i	14.79 ± 0.32^c	4.90 ± 0.12^g	12.18 ± 0.62^l
	W	8.98 ± 0.43^m	5.91 ± 0.36^d	8.72 ± 0.91^i	5.42 ± 0.29^e	31.10 ± 1.41^b
Kibatsi	W:SCG	17.23 ± 0.12^i	5.41 ± 0.27^h	10.93 ± 0.31^f	5.40 ± 0.11^f	26.60 ± 2.29^e
	W:SCG + LR	18.11 ± 0.17^h	5.02 ± 0.11^l	15.16 ± 0.98^b	4.40 ± 0.28^j	19.64 ± 0.99^g
	W	8.48 ± 0.45^k	6.12 ± 0.11^c	7.39 ± 0.45^l	5.53 ± 0.34^c	30.30 ± 0.82^d
Nyaruka	W:SCG	18.5 ± 0.31^g	5.62 ± 0.35^f	8.9 ± 0.81^h	5.42 ± 0.19^e	19.01 ± 2.21^i
	W:SCG + LR	14.06 ± 0.47^j	5.23 ± 0.31^j	19.76 ± 0.27^a	4.50 ± 0.17^i	18.08 ± 1.07^j
	W	9.40 ± 0.61^l	6.14 ± 0.78^b	7.65 ± 0.18^j	5.53 ± 0.23^c	30.50 ± 0.98^c
Reberaho	W:SCG	19.06 ± 0.3^f	5.55 ± 0.25^g	10.45 ± 0.11^g	5.51 ± 0.12^d	22.90 ± 1.27^f
	W:SCG + LR	19.45 ± 0.29^e	5.12 ± 0.21^k	13.3 ± 0.25^d	4.60 ± 0.36^h	19.23 ± 1.01^h

Values are means ± SD of 3 replications. Means with different superscripts within a column are significantly different at $p \leq 0.05$. W: whole wheat flour (control flour); W: SCG: whole wheat flour supplemented with spent coffee grounds (SCG); W: SCG+LR: whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L), and juice of rosemary leaves (R). Dough was fermented for 60 min.

reported that acrylamide concentration was low in the biscuit containing quinoa flour because the latter had much lower level of asparagine amino acid in relation to that of wheat flour.

Furthermore, Dough (Gihundo W: SCG+LR) with the highest antioxidant capacity (24.9 mg 100 g^{-1}) produced bread (Gihundo W: SCG+LR) with the lowest acrylamide content (10.5 μg kg^{-1}) (Table 2). The increased antioxidant capacity (8.48 to 24.9 mg 100 g^{-1}) of doughs by added SCG (25.84 mg 100 g^{-1}) and R (26.77 mg 100 g^{-1}) in comparison to those of control whole wheat doughs (8.48-10.88 mg 100 g^{-1}) may also have contributed to the low acrylamide formation in whole breads. These findings were also reported by Rwubatse [30].

The outcomes are consistent with a study that examined the impact of antioxidants on the development of acrylamide in bread and found that adding 1% of rosemary extract reduced acrylamide levels by 57–67%, depending on the kind of extract used [16, 18]. Additionally, antioxidants found in SCG were thought to reduce acrylamide amounts in biscuits in a study on the use of spent coffee grounds as a food additive in bakery goods [13–15].

On the other hand, the reduction of dough pH seemed to help also reduce acrylamide contents in whole breads as the acidic potential (2.70 pH) of lemon fruit juice (Table 2) lowered the pH of doughs below 5.41 where acrylamide formation is encountered. This pH was reduced from 5.91-6.22 (pH for whole wheat flour) to 5.02-5.83 (pH for doughs) (Table 2). It was explained that the initial amino-carbonyl reaction might be hampered by the protonation of the amino group because only the nonprotonised form of asparagine can form the Schiff base when pH is lower than the asparagine amino group's isoelectric point (5.41), thereby decreasing the Maillard reaction and acrylamide content in bread [35].

TABLE 3: Effect of fermentation duration on acrylamide reduction in whole wheat breads.

Wheat variety	Whole flours +ingredients	Fermentation duration (min)	Free asparagine content (mg 100 g^{-1})	Antioxidant capacity (mg 100 g^{-1})	Free asparagine content (mg 100 g^{-1})	Antioxidant capacity (mg 100 g^{-1})	Acrylamide content (μg kg^{-1})
			Whole doughs		Whole breads		
Gihundo	W	60	0.097 ± 0.004^a	10.88 ± 0.67^q	0.050 ± 0.001^f	7.48 ± 0.59^v	47.23 ± 0.92^a
	W:SCG		0.098 ± 0.004^a	22.12 ± 0.41^d	0.051 ± 0.002^f	11.60 ± 0.43^l	16.90 ± 0.80^q
	W:SCG+LR		0.091 ± 0.005^b	23.10 ± 0.38^c	0.038 ± 0.002^d	14.79 ± 0.96^f	12.18 ± 0.62^v
	W	120	0.065 ± 0.03^g	12.20 ± 0.18^p	0.028 ± 0.001^b	8.510 ± 0.92^t	44.01 ± 2.02^b
	W:SCG		0.066 ± 0.003^g	24.20 ± 0.92^b	0.029 ± 0.001^b	13.10 ± 0.42^i	13.12 ± 0.36^t
	W:SCG+LR		0.054 ± 0.001^j	24.90 ± 0.21^a	0.024 ± 0.001^a	16.39 ± 1.08^b	10.50 ± 2.27^w
Kibatsi	W	60	0.091 ± 0.003^b	8.98 ± 0.33^v	0.051 ± 0.002^f	8.72 ± 0.43^s	31.10 ± 1.41^c
	W:SCG		0.091 ± 0.004^b	17.23 ± 0.48^n	0.051 ± 0.002^f	10.93 ± 0.91^m	26.60 ± 2.29^h
	W:SCG+LR		0.073 ± 0.004^e	18.11 ± 0.21^m	0.033 ± 0.001^c	15.16 ± 0.83^d	19.64 ± 0.99^k
	W	120	0.062 ± 0.003^h	10.27 ± 0.40^s	0.029 ± 0.001^b	9.89 ± 0.43^o	29.50 ± 1.17^e
	W:SCG		0.061 ± 0.002^h	19.01 ± 0.11^k	0.051 ± 0.002^f	12.34 ± 1.09^j	23.23 ± 0.62^i
	W:SCG+LR		0.049 ± 0.001^k	20.60 ± 0.77^g	0.024 ± 0.001^a	14.74 ± 0.47^g	10.60 ± 0.51^w
Nyaruka	W	60	0.078 ± 0.004^d	8.48 ± 0.89^w	0.049 ± 0.002^{ef}	7.39 ± 1.01^w	30.30 ± 0.82^e
	W:SCG		0.077 ± 0.003^d	14.06 ± 0.59^o	0.048 ± 0.002^{ef}	8.90 ± 0.11^p	19.01 ± 2.21^n
	W:SCG+LR		0.069 ± 0.004^f	18.50 ± 0.61^l	0.038 ± 0.001^d	19.76 ± 0.45^a	18.08 ± 1.07^o
	W	120	0.057 ± 0.004^i	9.29 ± 0.28^u	0.028 ± 0.001^b	8.92 ± 0.29^q	27.24 ± 2.34^g
	W:SCG		0.077 ± 0.003^d	20.42 ± 0.19^f	0.029 ± 0.001^b	9.80 ± 0.34^o	14.41 ± 2.12^s
	W:SCG+LR		0.045 ± 0.001^l	20.71 ± 0.17^e	0.024 ± 0.001^a	15.04 ± 0.77^e	15.90 ± 1.11^r
Reberaho	W	60	0.085 ± 0.004^c	9.40 ± 0.23^t	0.046 ± 0.002^e	7.65 ± 0.91^u	30.50 ± 0.98^d
	W:SCG		0.086 ± 0.004^c	19.06 ± 0.45^j	0.046 ± 0.002^e	10.45 ± 0.98^n	22.90 ± 1.27^j
	W:SCG+LR		0.073 ± 0.003^e	19.45 ± 0.34^i	0.038 ± 0.001^d	13.30 ± 0.21^h	19.23 ± 1.01^m
	W	120	0.054 ± 0.002^j	10.58 ± 0.33^r	0.030 ± 0.002^{bc}	8.89 ± 0.36^r	27.20 ± 2.23^g
	W:SCG		0.085 ± 0.003^c	20.01 ± 0.47^{hi}	0.030 ± 0.001^{bc}	11.74 ± 0.12^k	12.34 ± 1.22^u
	W:SCG+LR		0.049 ± 0.002^k	20.09 ± 0.21^h	0.024 ± 0.005^a	15.72 ± 0.88^c	17.09 ± 1.15^p

Values are means ± SD of 3 replications. Means with different superscripts within a column are significantly different at $p \leq 0.05$. W: whole wheat flour (control flour); W: SCG: whole wheat flour supplemented with spent coffee grounds (SCG); W: SCG+LR: whole wheat flour supplemented with spent coffee grounds, juice of lemon fruits (L), and juice of rosemary leaves (R).

It was shown that all breads made from doughs fermented for 60 min had significantly greater acrylamide concentrations than breads made from doughs fermented for 120 minutes. This was caused by the fact that free asparagine was present in much lower concentrations in doughs fermented for 120 min than in doughs fermented for 60 minutes. Benedito De Barber et al. [36] and Fredriksson et al. [24] also noted a greater drop in asparagine contents as a result of extending the fermentation length. Asparagine may have been depleted during fermentation because yeasts utilised it as a source of nitrogen for their metabolic activity.

Compared to doughs fermented for 60 min without SCG or SCG+LR added, doughs fermented for 120 min containing SCG or SCG+LR produced breads with lower levels of acrylamide (Table 3). This showed that 120 min of fermentation assisted SCG and R in strengthening more the antioxidant capacity of doughs to prevent the formation of acrylamide in breads [30]. As a result, there was a substantially greater reduction of acrylamide formation in whole wheat breads due to the combined action of extended fermentation duration and added ingredients.

4. Conclusion

Four wheat varieties (Gihundo, Kibatsi, Nyaruka, and Reberaho) grown at the same location and under the same agroecological conditions were used to make whole breads. Whole bread (with no ingredients added) from the Gihundo variety had the highest content of acrylamide. The results showed that acrylamide formation in whole wheat bread was directly proportional to asparagine level of wheat variety.

The incorporation of spent coffee grounds (SCG), rosemary juice (R), and lemon juice (L) in doughs reduced significantly ($p < 0.05$) the acrylamide formation in control breads. Dough (Gihundo W: SCG+LR) with the highest antioxidant capacity produced bread (Gihundo W:SCG+LR) with the lowest acrylamide content. The low pH of doughs containing lemon fruit juice also helped reduce acrylamide content in

whole breads. The acrylamide contents were significantly higher in all breads from doughs fermented for 60 min than those of the breads from dough fermented for 120 min.

Whole wheat breads experienced a significantly larger decrease in acrylamide contents as a result of the combined effects of an extended fermentation time and incorporated SCG, L, and R.

Data Availability

The data used to support the findings of this study are openly available from the publishing journal.

Disclosure

Some of the results in the current paper are part of my PhD thesis, which is in the repository of the University of Nairobi, Kenya.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Contributions

Bernard Rwubatse was responsible for the conceptualization, original draft, methodology, and data curation. Michael Wandayi Okoth was responsible for the supervision, resources, validation, review, and editing. Angela Adhiambo Andago was responsible for the supervision, conceptualization, review, and editing. Sophia Ngala was responsible for the supervision, review, and editing. Clement Bitwayiki was responsible for the supervision, validation, review, and editing. Vedaste Ndungutse was responsible for the formal analysis. Jean Baptiste Ndahetuye was responsible for the software and formal analysis.

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