Oligosaccharide and antinutrient content of whole red haricot bean fermented in salt–sugar and salt-only solutions

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Abstract
Common beans (Phaseolus vulgaris L.) are nutritious and confer numerous health benefits. However, they are also high raffinose family oligosaccharides (RFOs) and antinutrients. Appreciable amounts of RFOs and antinutrients remain after soaking and cooking, causing flatulence and lowered mineral bioavailability to bean consumers. Fermentation has been shown to lower RFOs and antinutrients in bean flours and milk. However, beans are majorly consumed as whole grains. The purpose of this study was to develop a protocol for fermenting whole common beans. We fermented boiled whole red haricot beans and evaluated their effect on RFOs, tannins, and phytates. A factorial research design was used. Beans were sorted, soaked for 15 h, and boiled for 1 h. The beans were then fermented in 2% salt–sugar solution (SSF) and 2% salt-only solution (SOF) for 120 h. Microbial growth and pH were monitored every 24 h during fermentation. After fermentation, the beans were dried, milled, and the flours subjected to biochemical analysis. Fermentation favored the growth of lactic acid bacteria (LAB), lowering the pH to 3.88 and 5.26 in SSF and SOF batches, respectively. Tannin content reduced significantly by 64.70% and 73.19% in the SSF and SOF batches, respectively. Phytates reduced by 58.88% and 68.85%, respectively. Raffinose reduced significantly by 96.40% and 95.01%, respectively, whereas stachyose reduced by 95.92% and 94.11%, respectively. The highest reduction of antinutrients and RFOs occurred between 24 and 72 h of fermentation. Higher antinutrient losses occurred in the SOF batch, whereas higher RFO losses occurred in the SSF batch.

KEYWORDS
antinutrients, common bean, fermentation, raffinose family oligosaccharides

1 | INTRODUCTION

Common bean (Phaseolus vulgaris L.) is one of the five species of the family Phaseolus cultivated worldwide (Audu & Aremu, 2011; Berrios et al., 2010; Celmeli et al., 2018) East Africans are among the highest producers and consumers of common beans in sub-Saharan Africa (Díaz et al., 2010; Granito & Alvarez, 2006; Hirpa et al., 2015; Nakitto et al., 2015; Ugen et al., 2009) Common beans are rich in proteins, carbohydrates, vitamin B, soluble dietary fiber, minerals, and phytochemicals (Agarwal, 2016; Bautista-Expósito et al., 2018; Ganesan & Xu, 2017; Reyes-Bastidas et al., 2010; Suárez-Martínez et al., 2016). They are also known to stabilize blood sugar (Ganesan & Xu, 2017; Martín-Cabrejas et al., 2004), lower blood cholesterol (Martín-Cabrejas et al., 2004), reduce heart diseases (Chaudhary &...
Despite their nutritional and health benefits, consumption of common beans is associated with flatulence (Apata, 2008; Granito & Alvarez, 2006). A study by Agbenorhevi et al. (2010) reported that as a result of the anticipated negative effects, some people opt to avoid beans completely. The flatulence causing factor in common beans is the raffinose family oligosaccharides (RFOs) (Agbenorhevi et al., 2010; Ramadan, 2012). These are sugars with α-D-1,6-galactosidic linkages that are hydrolyzed by enzyme α-galactosidase (Berrios et al., 2010; Kumar et al., 2012). Unfortunately, this enzyme is absent in the human digestive system (Devindra et al., 2012; Saraswathy & Sadasivam, 2010; Sumarna, 2008). The RFOs, therefore, pass into the colon without being digested. The bacteria in the colon ferment them to produce hydrogen, carbon dioxide, and methane gases (Adewumi & Odunfa, 2009; Agarwal, 2016; Agbenorhevi et al., 2010; Apata, 2008; Berrios et al., 2010; Djaafar et al., 2013; Granito et al., 2003; Granito & Alvarez, 2006; Kumar et al., 2012; Ramadan, 2012). In some cases, these may be accompanied by diarrhea, headache, or dyspepsia (Adewumi & Odunfa, 2009).

Additionally, common beans contain tannins and phytates which are antinutritional compounds. Phytates lower mineral bioavailability by chelating minerals to form complex salts (Akande et al., 2010; Samtiya et al., 2020). Its presence in food impairs the utilization of phosphorous, iron, calcium, magnesium, manganese, copper, and zinc (Agarwal, 2016; Fernandes et al., 2010; Gemede & Ratta, 2014; Olaleye et al., 2020). Tannins precipitate proteins through hydrogen bonding and hydrophobic interactions, decreasing their digestibility (Akanle et al., 2010; Fernandes et al., 2010; Ferreira et al., 2019). Tannins also interfere with digestion by binding to digestive enzymes (Admassu, 2008; Akande et al., 2010; Boath et al., 2012; Díaz et al., 2010; Starzyńska-Janiszewska et al., 2014).

Fermentation is a simple and inexpensive processing technique (Ejigui et al., 2005; Martin-Cabrejas et al., 2004) that can be used at the household level. It causes biochemical modification of food through microbial activity (Dhull et al., 2021; Onwurafo et al., 2014; Samtiya et al., 2021). It also provides optimum pH conditions for enzymatic degradation of antinutrients in foods (Chelule et al., 2010; Porres et al., 2003; Roubos-van den Hil & Nout, 2011; Samtiya et al., 2020). This way fermentation improves the nutritional quality (Akpor & Aderije, 2014; Bourdichon et al., 2012; Ferreira et al., 2019; Granito & Alvarez, 2006), increases shelf-life (Chelule et al., 2010), and improves the flavor and texture of foods (Adinran et al., 2013; Martin-Cabrejas et al., 2004; Ongol, 2012).

Most people presoak and thermally process beans before consumption. However, antinutrients like tannins, phytates, and oligosaccharides are heat stable (Sozer et al., 2019). Therefore appreciable amounts of these antinutrients are left in the beans after heat treatment. Further reduction of RFOs and antinutrients is therefore desirable (Starzyńska-Janiszewska et al., 2014) in order to improve the nutritional value of legumes. This research, therefore, aimed to develop a fermentation protocol for whole red haricot bean.

# 2 | MATERIALS AND METHODS

## 2.1 | Materials

Raw red haricot beans (Wairimu) were acquired from the National Cereals and Produce Board (NCPB) Nairobi, Kenya. The table salt (Kensalt) and local sugar (Kabras) used in this study were purchased locally. The media used for microbial determination was analytical grade from Himedia, Mumbai, India.

## 2.2 | Methods

### 2.2.1 | Sample preparation

The red haricot beans were hand-sorted to remove dirt and defective grains. About 1.5 kg of the sorted red haricot beans were washed in distilled water and all floats removed. The beans were then soaked in distilled water at a ratio of 1:5 weight per volume (w/v) for 15 h at room temperature. The soaking water was discarded and beans rinsed in distilled water. About 200 g of the soaked beans was transferred to seven experiment bottles. The fermentation bottles were then topped up with 400 ml of distilled water (1:2 w/v). The bottles were put on a hot plate and the beans boiled for 60 min. The boiling water was then drained and the beans allowed to cool in the bottles before fermentation. Boiled beans from one bottle were removed from the bottle, rinsed in distilled water, and then dried at 60°C for 10 h in an oven. The dried boiled beans were then milled with a steel mill then stored in a freezer awaiting biochemical analysis (boiled sample).

### 2.2.2 | Preparation of fermentation solutions

The concentration of fermentation solution used in this study was informed by our previous work (Kitum et al., 2018). Fermentation solution (2% salt–sugar solution) was prepared by dissolving 12 g of salt and 12 g of sugar (1:1 w/w) in three fermentation bottles containing 600 ml of distilled water each. Fermentation solution (2% salt-only solution) was prepared by dissolving 12 g of table salt in three fermentation bottles containing 600 ml of distilled water each. The bottles with the fermentation solutions were sterilized by autoclaving at 121°C for 15 min then allowed to cool to room temperature before use.

### 2.2.3 | Fermentation of boiled beans

The sterile fermentation solution from above was transferred to the experiment bottles with cooled boiled red haricot beans. The six bottles were labeled 24 h salt–sugar solution (SSF), 72 h SSF, 120 h SSF, 24 h salt-only solution (SOF), 72 h SOF, and 120 h SOF. The fermentation bottles were then left on a sterile bench to ferment at 25 ± 2°C for 120 h. Fermentation solution samples were drawn aseptically.
using a pipette and sterile pipette tips at 0, 24, 72, and 120 h for pH determination and microbial enumeration.

2.2.4 | pH determination

Approximately 5 ml of the fermentation solution was drawn aseptically after every 24 h of fermentation for pH determination using a pH meter (HI 2211, Hanna Instruments, Japan).

2.2.5 | Microbial enumeration

Conventional microbiological methods were used for microbial enumeration. The 5 ml of the fermentation solution were transferred into a sterile 9-ml test tube and vortexed properly to mix. About 1 ml of the mixture was transferred to a 9-ml test tube containing quarter-strength Ringer’s solution to make serial dilutions. Exactly 10 μl of the aliquots from different dilutions were transferred to Petri dishes containing de Man, Rogosa, and Sharpe (MRS) agar for lactic acid bacteria (LAB) enumeration; violet red bile agar (VRBGA) for enterobacteria enumeration; plate count agar (PCA) for total aerobic bacteria enumeration; and potato dextrose agar (PDA) for yeast and molds enumeration. They were then spread plated followed by incubation of the agar plates at respective temperatures. Each analysis was carried out in triplicate. All bacterial and fungal counts were expressed as colony-forming units per milliliter (CFU/ml).

2.2.6 | Sample preparation for biochemical tests

At the end of each fermentation, the fermentation solution was discarded and the fermented red haricot bean (P. vulgaris L) spread on clean trays. They were then dried in an oven at 60°C for 10 h. The dried beans were then milled and the resulting ample flour stored at 4°C in zip lock bags awaiting biochemical analysis.

2.2.7 | Determination of RFOs content on a dry weight basis (DWB)

Extraction of raffinose and stachyose was done using the method of Antonio et al. (2008) with adjustments. About 0.01 g of the raw and processed bean flours was weighed into microcentrifuge tubes containing 250-μl ice-cold chloroform:methanol (3:7 v/v). The contents were then vortexed in a vortex (Iwaki mixer, TM-151, Japan) for 1 min and the mixture incubated at -4°C for 2 h to stop metabolism and extract water-soluble metabolites. After incubation, 200 μl of ice-cold water was added, and the tubes were shaken for 30 min in a shaker (Ika Labortechnik, KS-250 B, Germany). The samples were then topped up to 1000 μl with cold distilled water and centrifuged in a microcentrifuge (Genevac, DNA-23050-A00, England) at 17,900 × g, at 4°C for 10 min. The upper phase was transferred into sample vials (2.0 ml) followed by liquid chromatography (LC) ion trap mass spectrometric analysis in an LC–mass spectrophotometry (MS) machine (Genevac, DNA-23050-A00, England).

2.2.8 | Determination of tannin content (DWB)

Tannin content was determined using the vanillin–HCL method of Price et al. (1978). About 0.2 g of bean flours was weighed into centrifuge tubes. About 10 ml of 4% methanolic HCL was then added into each of the tubes and then shaken for 20 min on a shaker. The sample mixtures were then centrifuged at 2500 rotations per minute (rpm) for 10 min in a centrifuge (Hettich, D-78532 Tuttlingen, Germany). The supernatants were transferred into 25-ml volumetric flasks. About 5 ml of 1% methanolic HCL was then added to the precipitate in the centrifuge tubes and centrifuged as described above. The supernatants were then transferred into the 25-ml volumetric flasks above and topped up to the mark using 1% methanolic HCL. About 1 ml of the supernatants was then transferred into two test tubes each. To one test tube, 5 ml of freshly prepared mixed reagent (8% methanolic HCL + 4% vanillin in methanol) was added. To the other test tube, 5 ml of 4% methanolic HCL was added. A series of catechin standards were prepared and 1 ml of each of the standard concentrations transferred into a test tube and 5 ml of the mixed reagent added. The sample extracts and standards were allowed to sit for 20 min for color development. The absorbance of the sample extracts and standard solutions were read at 500 nm using a UV-vis photospectrophotometer (UV mini 1240 model, Shimadzu, Japan).

2.2.9 | Determination of phytate content (DWB)

Phytates were extracted using the method of Camire and Clydesdale (2006) with modification. About 0.5 g of milled bean flours was extracted. LC–MS (Genevac, DNA-23050-A00, England) analysis was done using Shimadzu Refractive Index Detector (RID 6A). The mobile phase was 0.005 N sodium acetate in distilled water at a flow rate of 0.5 μl/min.

2.3 | Statistical analysis

Each analysis was done in triplicate and the experiments conducted three times. Data were presented as means ± standard error of means (SEM) or standard deviation (SD) of three separate determinations. Contrast ANOVA was conducted and pairwise comparison of estimated marginal means at P ≤ 0.05 using least significant difference (LSD). Statistical analysis was carried out using SPSS statistics version 23.
3  | RESULTS

3.1  | Microbial growth

At the start of fermentation of boiled red haricot bean, LAB, total aerobic count (TAC), coliform, yeast, and mold were not detected in both the SSF and SOF batches. This is in agreement with the findings of Ulloa et al. (2015). However, after 24 h of fermentation, significant growth \( (P < 0.05) \) in LAB count and TAC occurred. The LAB counts were at \( \log_{10} 5.6 \) and \( \log_{10} 6.3 \) in the SOF and SSF batches, respectively, whereas the TAC reached \( \log_{10} 3.5 \) and \( \log_{10} 4.0 \), respectively. The LAB counts increased with an increase in fermentation time in both batches. At the end of fermentation (120 h), the LAB count in the SSF batch was at \( \log_{10} 8.5 \). However, in the SOF batch, there was no significant change \( (P > 0.05) \) in LAB count after 72 h of fermentation \( (\log_{10} 7.4) \). With increased fermentation time, TAC continued to increase significantly \( (P < 0.005) \) in both the SOF and SSF batches. At the end of fermentation (120 h), TACs in SOF and SSF batches were at \( \log_{10} 8.6 \) and \( \log_{10} 9.4 \), respectively. Throughout the fermentation, no coliform, yeast, or mold was detected in both the SOF and SSF batches.

3.2  | pH

The changes in pH of the fermentation solutions during the fermentation of boiled whole red haricot bean are presented in Tables 1 and 2. The pH of the fermentation solution at the beginning of fermentation was 6.06. A decrease in the pH was observed with increased fermentation time. In the SOF batch, the pH decrease was slow at the end of the fermentation; the pH was 5.26 (Table 2). In the SSF batch, a significant decrease \( (P < 0.05) \) to 4.8 was observed after 24 h of fermentation (Table 1). The pH continued to decrease significantly with an increase in fermentation time recording a pH of 3.88 after 120 h.

3.3  | Raffinose family oligosaccharides

The effect of fermentation of boiled whole red haricot bean on raffinose concentration is presented in Figure 1. The presoaked boiled beans had a raffinose concentration of 50.30 mg/100 g. Fermentation of the beans in SSF reduced raffinose concentration to 11.86 mg/100 g (76.42% decrease). Increased fermentation time resulted in a further decrease in the raffinose concentration. At the end of 120 h of fermentation, the raffinose concentration had decreased by 96.40% to 1.81 mg/100 g.

In the SOF batch, the raffinose concentration decreased by 48.5% after 24 h of fermentation to 25.9 mg/100 g. However, after 72 h of fermentation, the raffinose content decreased significantly by 81.96% to 4.67 mg/100 g. After 120 h of fermentation, the raffinose concentration in the SOF batch reduced by 95.01% to 2.51 mg/100 g. The decrease in raffinose concentration as a result of fermentation in this current study is in agreement with that of Adewumi and Odunfa (2009).

The effect of fermentation of boiled whole red haricot bean \( (P. vulgaris L.) \) on stachyose is presented in Figure 2. Stachyose concentration of presoaked boiled red haricot bean was 995.76 mg/100 g. Fermentation reduced stachyose concentration

| Fermentation time | pH         | LAB        | TAC        | Yeast and molds | Coliforms |
|-------------------|------------|------------|------------|----------------|-----------|
| 0 h               | 6.05 ± 0.01a | ND         | ND         | ND             | ND        |
| 24 h              | 4.69 ± 0.15b | 6.32 ± 0.02a | 4.06 ± 0.08a | ND             | ND        |
| 72 h              | 4.01 ± 0.01a | 7.85 ± 1.17b | 8.30 ± 0.02b | ND             | ND        |
| 120 h             | 3.88 ± 0.01a | 8.58 ± 0.31b | 9.40 ± 0.02c | ND             | ND        |
| \( P \) value     | <0.0001    | <0.0001    | <0.0001    | -              | -         |

Note: Values are \( \log_{10} \) means of triplicate determinations ± SD. Means in the same column followed by the same subscript are not significantly different, \( P > 0.05 \). Mean comparison for the treatments was done using the LSD test. Abbreviations: LAB, lactic acid bacteria; LSD, least significant difference; ND, not detected; TAC, total aerobic count.

| Fermentation time | pH         | LAB        | TAC        | Yeast and molds | Coliforms |
|-------------------|------------|------------|------------|----------------|-----------|
| 0 h               | 6.06 ± 0.01b | ND         | ND         | ND             | ND        |
| 24 h              | 5.75 ± 0.36b | 5.62 ± 0.01a | 3.48 ± 0.21a | ND             | ND        |
| 72 h              | 5.76 ± 0.01b | 7.40 ± 0.29b | 7.74 ± 0.06b | ND             | ND        |
| 120 h             | 5.26 ± 0.02a | 7.42 ± 0.01a | 8.58 ± 0.15a | ND             | ND        |
| \( P \) value     | 0.001      | <0.0001    | <0.0001    | -              | -         |

Note: Values are \( \log_{10} \) means of triplicate determinations ± SD. Means in the same column followed by the same subscript are not significantly different, \( P > 0.05 \). Mean comparison for the treatments was done using the LSD test. Abbreviations: LAB, lactic acid bacteria; LSD, least significant difference; ND, not detected; TAC, total aerobic count.
significantly \( (P < 0.05) \) in both the SSF and SOF batches. This is in agreement with the findings of Adewumi and Odunfa (2009) and Granito et al. (2003) who reported a decrease in stachyose concentration as a result of fermentation of Vigna unguiculata beans and common beans (\textit{P. vulgaris}), respectively. After 24 h of fermentation, stachyose concentration in the SSF batch decreased by 79.48% to 204.34 mg/100 g. The stachyose concentration continued to decrease with an increase in fermentation time. At the end of fermentation (120 h), the stachyose concentration in the SSF batch had decreased by 95.92% to 40.61 mg/100 g.

In the SOF batch, stachyose concentration decreased by 63.69% to 361.57 mg/100 g after 24 h of fermentation. A further decrease of 72.02% and 41.92% occurred after 72 h and 120 h of fermentation. At the end of fermentation (120 h), stachyose concentration in the SOF batch had decreased by 94.11% to 58.76 mg/100 g.

### Tannins

The effect of fermentation of boiled whole red haricot bean \((P. vulgaris \text{ L.})\) on tannin concentration is presented in Figure 3. The tannin concentration of the presoaked boiled beans was 274.77 mg/100 g. Fermentation resulted in a significant decrease \((P < 0.05)\) in tannin concentration in both SSF and SOF batches. This is in agreement with Granito and Alvarez (2006) and Adeniran et al. (2013) who reported an 83%, 89.5%, and 68.42% decrease in tannin content of cooked fermented black beans, lima bean, and locust bean, respectively. Tannin concentration losses were highest within the first 24 h of fermentation. The tannin concentration decreased from 274.76 mg/100 g to 181.90 mg/100 g and 148.89 mg/100 g in the SSF and SOF batches, respectively. With increased fermentation time, the tannin concentration in the SSF and SOF batches continued to decrease. At the end of 120 h of fermentation, the tannin concentration in these batches was 97.07 mg/100 g (64.70%) and 73.67 mg/100 g (73.19%), respectively. Throughout fermentation, tannin concentration in the SOF batch was lower than in the SSF batch.

### Phytates

The effect of fermentation on the phytate concentration of boiled whole red haricot bean is presented in Figure 4. The phytate concentration in presoaked boiled red haricot bean was 350.11 mg/100 g. Fermentation of the beans resulted in a reduction of phytate concentration. This was in agreement with the findings of Adeniran et al. (2013) who reported a 77.82% and 73.53% reduction of phytates in lima and locust beans, respectively. In the SSF batches, the highest reduction of phytate concentration occurred after 24 h of fermentation. This reduced the phytate concentration from 350.11 mg/100 g to 157.09 mg/100 g and 145.65 mg/100 g after 72 h and 120 h of fermentation. At the end of fermentation (120 h), the phytate concentration in the SSF batch was lower than in the SOF batch.

A similar trend was observed in the SOF batch, the highest reduction of phytate concentration from 350.11 mg/100 g to 147.40 mg/100 g occurred after 24 h of fermentation. Increased
fermentation time resulted in a further decrease in phytate concentration to 122.04 mg/100 g and 109.07 mg/100 g at the end of 72 and 120 h of fermentation, respectively. At the end of fermentation, the SOF batch had the lowest phytate concentration compared with the SSF batch.

4 | DISCUSSION

Heat treatment of the beans through boiling lowered the LAB, TAC, yeasts and molds, and coliforms to undetectable levels. However, after 24 h of fermentation, LAB and TAC growth occurred in both batches. Tope (2013) reported findings similar to this by isolating LAB and bacillus species during the fermentation of cooked lima bean. This could be attributed to the fact that boiling may not have completely eliminated the LAB and TAC but reduced them to undetectable levels (Haddaji et al., 2015). Haddaji et al., 2015 also reported that LAB has the ability to survive heat shocks. They produce proteins that enable them to survive under harsh conditions. Coliforms, yeasts, and molds were not detected throughout the fermentation process. This could be attributed to heat treatment and the acidic environment that inhibited their growth (Granito & Alvarez, 2006; Onwurafor et al., 2016) favorable for \( \alpha \)-galactosidase enzymes that break down the \( \alpha \)-1,6-glycosidic linkages (Adewumi & Odunfa, 2009). The presence of raffinose in the beans induces the production of \( \alpha \)-galactosidase (Carevic et al., 2016; Kumar et al., 2012). The highest losses of 76% and 79% in raffinose and stachyose, respectively, occurred after 24 h of fermentation in the SSF batch when pH was 4.8. In the SOF batch, the highest losses of 82% and 72% in raffinose and stachyose concentration occurred after 72 h of fermentation when pH was at 5.8. This correlated with the optimal time between 12 and 72 h (Kumar et al., 2012) and pH (Carevic et al., 2016) favorable for \( \alpha \)-galactosidase activity. Carevic et al. (2016) reported that acidic pH favored the activity of \( \alpha \)-galactosidase whereas an increase towards neutral pH reduced its activity. This could be the reason why although pH in the SOF batch was higher than 5.0, raffinose and stachyose concentration continued to reduce significantly to levels comparable with SSF. The decrease in RFOs concentration could also be attributed to the exposure of the RFO sugars to microbial \( \alpha \)-galactosidases as a result of boiling which softens and breaks the seed coat.

Presoaking of red haricot beans resulted in some losses of tannins. This is attributed to leaching out of tannins into soaking water. Fermentation of the beans lowered the tannin content significantly. This is a result of hydrolysis of polyphenolic compounds of tannin complexes (Adeniran et al., 2013). It was observed that tannin content decreased more in the SOF batch compared with the SSF batch. This could be attributed to the high pH 6.06–5.26 which favors the activity of enzyme tannase. Battestin and Macedo (2007) established that the optimal tannase activity was at pH 6.5. In the SSF batch, the pH had reduced significantly to 4.8 after 24 h of fermentation, therefore lowering tannase activity. In their findings, Battestin and Macedo (2007) reported a tannase activity of 45% at pH 3.5.

The reduction of phytic acid during fermentation could be attributed to the activity of phytase enzyme from the beans, fermenting microorganisms, or a combination of the two (Adeniran et al., 2013; Kocková et al., 2011; Ojokoh et al., 2013; Porres et al., 2003). The highest phytic acid loss occurred after 24 h of fermentation. This is in agreement with Rasha Mohamed et al. (2011) who reported 58.1% and 70.6% loss of phytate after 24 and 72 h of LAB fermentation of kidney beans, respectively. At this time, the pH range was between 6.06 and 6 in the SOF batch and 6.06 and 4.8 in the SSF batch (Tables 1 and 2). Ejigui et al. (2005) reported that optimum activity for cereal phytase is between 5.5 and 5.3. Zamudio and Gonza (2001) also reported that LAB had the highest extracellular phytase activity at pH 5.5. Increased fermentation time, resulted in a further significant decrease in the phytic acid content of the SOF batch. This could be attributed to its high pH throughout fermentation. Increased fermentation time did not cause any significant changes in phytic acid concentration in the SSF batch. This could be attributed to the low pH of ≤4 which could have denatured the phytase enzymes. Ejigui et al. (2005) also reported that in addition to phytase enzyme activity, phytic acid of some cereals can also be eliminated through passive diffusion of water-soluble phytase. This could therefore be the reason why some losses in phytic acid were observed in the SSF batch even after pH was below 5.1.

5 | CONCLUSION

The boiling of red haricot beans reduced all bacteria and yeast and molds to nondetectable levels. This provided a favorable
environment for LAB to dominate the fermentation of the beans, therefore making the fermented red haricot bean safe to consume. Production of organic acids by LABs during fermentation lowered the pH in both SSF and SOF batches. Low pH provides a conducive environment for microbial and endogenous enzymes to break down the raffinose, stachyose, tannins, and phytates in the beans. The highest reduction of tannins and phytates occurred during the first 24 h of fermentation. In the SSF batch, the RFOs had the highest reduction after 24 h of fermentation, whereas in the SOF, the highest reduction was after 72 h of fermentation. The highest reduction of each antinutrient coincided with the optimum pH of the enzyme responsible for its degradation. We, therefore, conclude fermentation is an effective method for reducing antinutrients and RFOs in whole red haricot bean.

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CONFLICT OF INTEREST
The authors declare no conflict of interest as a result of the publication of this paper.

ETHICS STATEMENT
Ethical principles governing research were adhered to. No animal or human subjects were used in this study. The red haricot bean used in this study is a common staple in Kenya and has no reported toxicity.

AUTHOR CONTRIBUTIONS
All four authors contributed to the conceptualization of the research topic. Vivian C. Kitum carried out the experimentation and analysis of data. All four authors contributed to the interpretation of data and writing, preview, and editing of the manuscript.

DATA AVAILABILITY STATEMENT
The analyzed data used to support the findings of this study are included within the article.

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