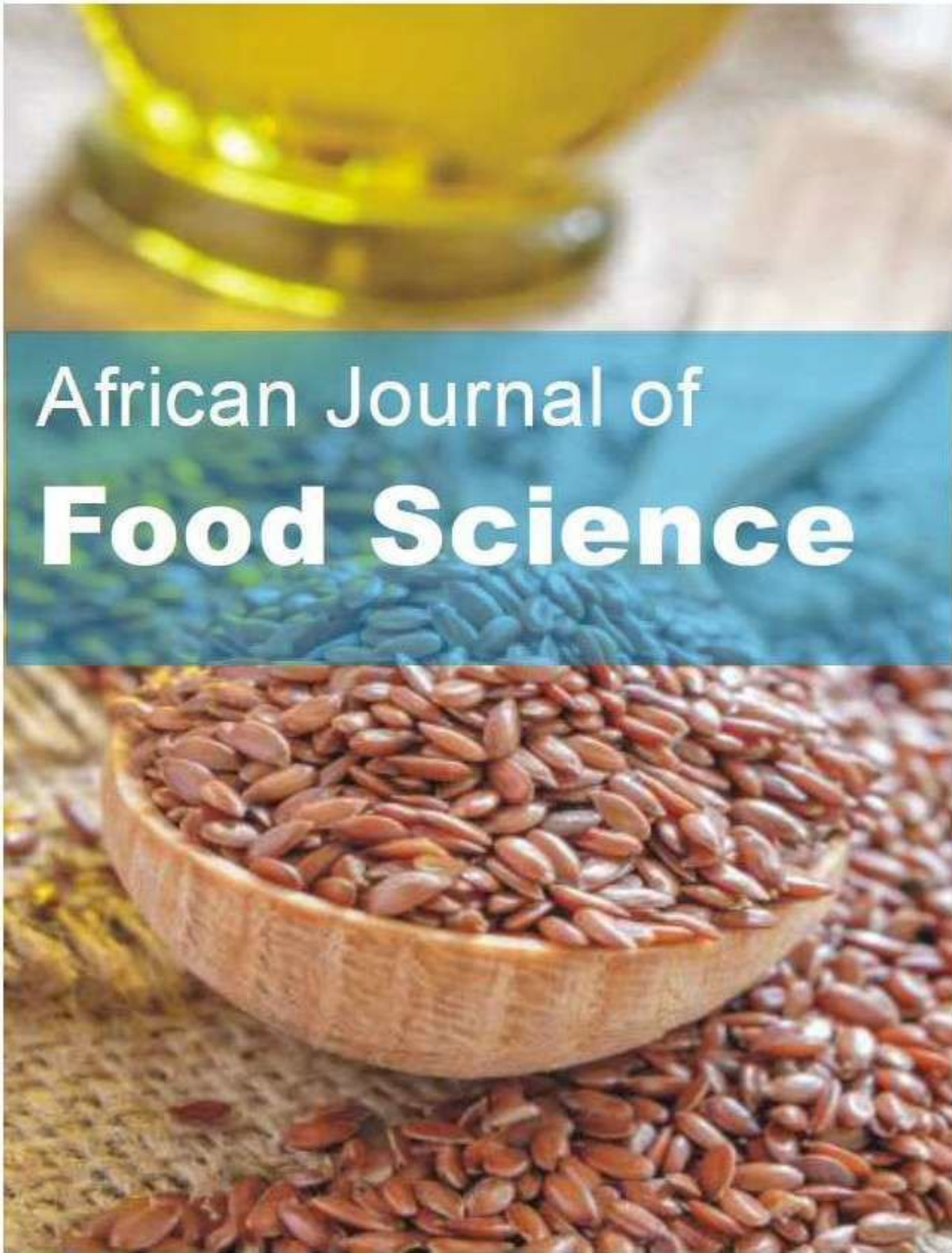


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Full Length Research Paper

Impacts of covid-19 measures on supply and distribution of milk on small-scale processors in Nakuru, Uasin Gishu, and Nyandarua Counties, Kenya

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COVID-19 poses serious threats to humanity and the world's economy, leading to disruptions of the food supply chain. The dairy processing industry was highly affected because of the perishability of its products and time-sensitivity of both the supply and distribution chain. The study therefore, aimed to assess the effects of COVID-19 containment measures on the supply and distribution of small-scale dairy processors in Nakuru, Nyandarua, and Uasin-Gishu counties, Kenya. A descriptive cross-sectional research design was used, where a total of 26 small-scale mini-dairies and cottages from the registered and licensed list provided by the Kenya Dairy Board were interviewed using semi-structured questions. Results showed that the primary processed dairy product was pasteurized milk (48%) followed by fermented milk and yoghurt (32%). Before and during COVID-19, there was a significant reduction in the quantity of milk processed by small-scale dairy companies ($P<0.05$), as well as in the number of employees ($P<0.05$) causing an increase in the company's processing costs and modification of strategies to continue daily milk collection and distribution. Therefore, policies and measures should occasionally be tightened or loosened depending on the spread of the virus to guarantee the resilience of the dairy supply and distribution chain during pandemic outbreaks.

Key words: COVID-19 pandemic, Food supply chain, Small-scale dairy processors.

INTRODUCTION

In Kenya, the dairy industry contributes about 14% GDP to the agriculture sector and 4% to the national GDP (KNBS, 2019; Kurgat et al., 2023). It is a significant source of income as well as employment with its activities from production, and processing to retail (Meseret et al., 2021). Kenya has a diverse dairy sector comprising both large scale commercial processors and small-scale

processors who operate at the grassroots level, primarily sourcing milk from small-holder farmers. The dairy industry however faces a lot of challenges limiting its expansion.

In 2020, the outbreak of the COVID-19 pandemic brought yet another challenge, not as a threat to humanity causing a lot of deaths but also to economies

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(Galanakis et al., 2020). The first outbreak in Kenya was reported on March 13th, 2020. The rapid spread of the virus led the government like that of most countries to implement strict measures to reduce the spread of the virus disease (Odhong et al., 2020). These measures included the border closures, travel bans, restrictions on public gatherings, school closures, market closures, and dark to dawn curfews were among the measures implemented, with agricultural products allowed to be transported during the day. Although such measures decreased disease spread, they crippled the economy and impacted a wide range of industries and business enterprises (Mutua et al., 2021). According to the Kenya Dairy Board, the country had witnessed substantial growth over the year, driven by increased demand and changing consumer preferences (KDB, 2021). In 2020, the country produced approximately, 5.2 billion litres of milk with an annual growth rate of around 4%. However, from the onset of the pandemic, GDP fell by 5.7% compared to its 4.9% growth before March 2020 (KNBS, 2020). The agriculture sector, on the other hand, suffered 3 million USD during the lockdowns despite its one-third contribution to the national GDP and it is being classified as an essential service and the agricultural products transported during the day (Mutua et al., 2021) with the dairy industry particularly, suffering a 63.4 million litres in January to 40.2 million litres in June marketed during the COVID-19 pandemic in 2020.

The global supply chain was affected by the COVID-19 pandemic. In China for instance, reports show that the most affected sub-sector of agriculture was dairy due to dairy products being highly perishable and their reliability on a supply chain that is dependent on time (Wang et al., 2020). Due to the pandemic and associated measures such as lockdowns, transportation restrictions and market closures (Ganesh-Kumar et al., 2020), small dairies could not sell their milk even at lower prices thus resulting in them dumping the milk (Acosta et al., 2021). Furthermore, the lockdowns and reduced mobility impacted milk collection and processing, leading to decreased production and increased wastage (Huang et al., 2021). In India, the closure of restaurants and institutions, a major market for dairy products, severely affected demand and led to surplus milk (Kumar et al., 2021). Similarly, in Romania, dairy processors faced challenges in procuring raw milk due to transportation restrictions (Bhat et al., 2022). Similarly in Canada and the United Kingdom, Weersink et al., (2021), reported that small dairy companies had to dump their milk because of the sharp decrease in dairy demand. In the USA, the closure of schools and a shift to remote learning affected the demand for milk and dairy products in the institutional sector (Acosta et al., 2021). In Ethiopia, there was a reduction in the volume of milk sold to processors due to fear that the disease could be transmitted via contaminated milk (Meseret et al., 2021), although COVID-19 is not transmitted through food or

packaging materials. Additionally, in Senegal, Burkina Faso, and Madagascar milk supply was hindered due to travel restrictions for dairy processors especially small-scale (Vall et al., 2021). Similarly (Barrett, 2020) described that the dairy value chain was constrained from operating to its full potential due to disruptions in the food systems.

Therefore, the objective of this research was to find out how the COVID-19 pandemic, in conjunction with strict measures, has directly or indirectly affected the dairy processing industry, and to aid in developing policy recommendations and industrial approaches that will improve the dairy industry's overall resilience to future pandemics.

MATERIALS AND METHODS

In this research work, the authors attempt to comprehensively understand how the COVID-19 pandemic measures affected the small-scale dairy processors in their supply and distribution of milk. Our research framework was as follows: First, we obtained a certificate of ethical clearance from NACOSTI. The Kenya Dairy Board then provided us with a list of existing dairy processors in the featured counties. We then conducted semi-structured interviews with each dairy processor's management teams about the impact of the COVID-19 pandemic on their operations, and coded the collected data for analysis, interpretation, and visualization. We then discussed how the supply and distribution of small-scale dairy processors was influenced before and during the COVID-19 pandemic. Afterwards that, we proposed relevant policy recommendations and improvements to be implemented to protect dairy processors in the event of future unprecedented pandemics.

Study area

The counties, (Nakuru and Uasin Gishu) in the old Rift Valley Province and Nyandarua in the old Central Province are situated in the highlands, where dairy-related activities—including production, processing, and consumption are most dominant (Mogotu et al., 2022; Otieno et al., 2020). Figure 1 shows the map of the study.

Uasin Gishu County

Uasin Gishu County is geologically located between longitudes 34° 50" to the East and 35° 37" to the West and latitudes 0° 03" to the South and 0° 55" to the North. The county is situated in the Midwest of Kenya's Rift Valley some 330 km North West of Nairobi. It borders Kericho County to the South, Nandi County to the South West, Bungoma County to the West, Trans Nzoia to the North, Elgeyo-Marakwet to the East, and Baringo County to the South East (Kurgat et al., 2019). The county is located on a plateau covering an area of 3345.2 Sq. Km with temperatures ranging from a minimum of 8.4°C to a maximum of 27°C. Uasin Gishu County has two rainy seasons with average rainfall from 900 mm to 1,200 mm per annum. The cool temperate climate favours mixed farming including maize, wheat, beef, and dairy farming and processing being dominant.

Nyandarua County

Nyandarua County is situated on coordinates latitude: 0°32' 59.99" North and longitude: 36°36' 59.99" East bordering Laikipia County to

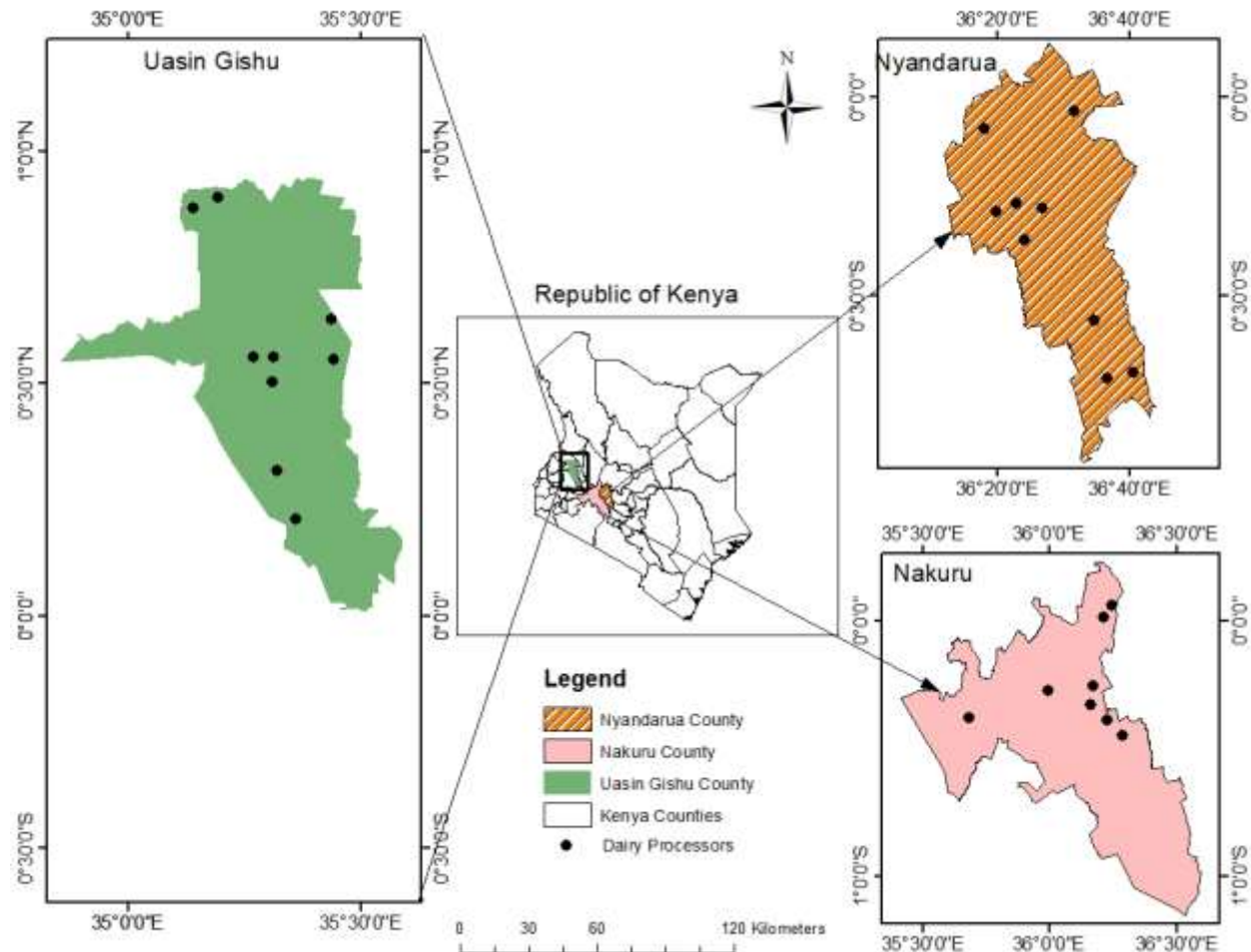


Figure 1. Map of the study area.
Source: Authors

the North, Nyeri, and Muranga counties to the East, Thika and Kiambu counties to the South and Nakuru county to the West. The county covers an area of 3,304 Sq.km (Muia et al., 2011). It falls within the central highlands with an altitude range of 2350 and 3000 meters above sea level with a mean temperature of 22 °C. The mean annual rainfall in the county is between 1000 mm and 2000 mm, occurring in two seasons. The long rains season occurs between March and June and the short rains season between October and December, agriculture is the main economic activity in the County with dairy production being dominant (Moturi et al., 2015).

Nakuru County

Nakuru County is situated in the Rift Valley, covering an area of 7,235.3 Sq. km. It is located between longitudes 35° 28" and 35° 36" and latitudes 0° 12" and 1° 10" South, lying about 2100 m above sea level. Nakuru borders eight other counties, namely Kericho and Bomet to the west, Koibatek and Laikipia to the north, Nyandarua to the east, Narok to the southwest, and Kajiado and Kiambu to the south. The county's economic foundation is agriculture, which is dominated by large-scale agricultural, horticulture, and dairy farming and processing.

Study design

The study involved a cross-section design whereby data was collected once at a specific point in time from April to June 2022.

Sampling of dairy processing companies

The mixed sampling technique was used, and the areas (Nakuru, Uasin Gishu, and Nyandarua counties) and the size of the processors (mini-dairies and cottage processors) were purposively selected. Then the processors were retrieved from the registered and licensed list from the Dairy Board of Kenya located in the counties. A total of 26 small-scale mini-dairies and cottages were available for the study whereby in Nakuru 8 out of 10 were selected, in Nyandarua 9 out of 11 were selected and in Uasin Gishu 9 out of 10 were sampled for the study.

Data collection

Face-to-face interviews were conducted with the managers of the small-scale dairy companies using two-part semi-structured open-ended and closed-ended questions. The first section of the

Table 1. Characteristics of the dairy processing companies analysed.

Parameter	Category	Frequency (n)	Percentage
Manager of the processing companies			
Gender	Male	22	84
	Female	04	16
Age	24-29	05	19
	30-35	7	27
	36-40	3	12
	40-45	7	27
	>45	4	15
Education level	Primary	02	8
	Secondary	01	4
	Craft certificate	01	4
	Diploma	12	48
	University	10	36
Number of employees	1-10	17	65
	10-49	9	35
Processing capacity (litres)	<4000	15	58
	4001-8000	07	27
	8001-10000	02	8
Training in food processing	Yes	24	92
	No	02	8
Quality management system	GMP	26	100
	HACCP	08	31

Source: Authors

questions comprised the general information including demographic characteristics such as gender, education level, age, and the general characteristics of the processing company involving with the policy documentation used to verify food safety performance, number of employees, and training on the food safety system in place in the plant. Whereas the second section inquired about the supply and distribution chain before and during COVID-19

Data management and analysis

The collected data were coded and then analysed using Statistical Package for Social Sciences (SPSS). Chi-square, paired comparison t-test, and descriptive statistical analysis were performed to obtain frequencies, percentages, and p-values. Finally, they were organized in tables.

Ethical consideration

A research permit was sought from the Vice Chancellor of Sokoine University of Agriculture. A research clearance permit and letter of authority were acquired from National Commission for Science, Technology, and Innovation (NACOSTI) and the Kenya Dairy Board to be used for data collecting. The respondents' disclosures of their

personal information were kept completely confidential. This was accomplished by utilizing the data without specifically specifying the individuals from whom it was acquired.

RESULTS AND DISCUSSION

Characteristics of the small-scale processing companies (mini-dairies and cottages)

From the 26 mini-dairies and cottages that participated in the study, it was found that majority of managers of the processing plants were male (84%) as compared to female (16%) aged between 30-45 years (52%) with 84% having a tertiary education level (that is 48% diploma and 36% degree) in Table 1. Additionally, the number of persons employed in the mini-dairies and cottages ranged from 1-10 staff members all of whom are reported to have received training on the quality management system in use at the company which all the twenty-six (26) mini-dairies and cottages reported the use of the GMP in their daily operations while (8) additionally

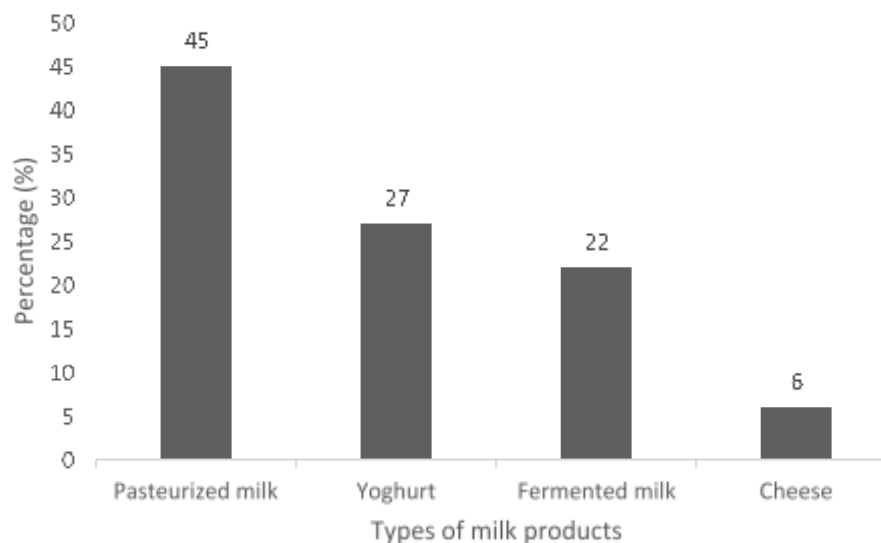


Figure 2. Types of milk products processed in the mini-dairies and cottages.
Source: Authors

Table 2. Quantity of milk received against the number of companies before and during the COVID-19 pandemic outbreak.

Litres of milk received in the company	Before COVID-19 n (%)	During COVID-19 n (%)	change (%)
<4000	15 (57)	20 (77)	+20
4001-8000	09 (35)	05 (19)	-16
8001-10000	02 (8)	01 (4)	-4

Source: Authors

implemented HACCP in their quality management system.

With respect to education level, majority (diploma 48%; University 36%) of the managers had tertiary education level. Through tertiary training, the managers are trained in various aspects related to quality management and processing. The knowledge is used to improve the handling and processing of milk and milk products in their respective companies.

The majority (65%) of processors were micro-scale enterprises with less than 10 employees. This is according to the Ministry of Industrialization, Trade and Enterprise Development (2020) categorisation, all companies having less than 10 employees are termed micro-enterprises; 10-49 employees are known as small enterprises whereas those with 50-99 are medium-sized enterprises.

As illustrated in Figure 2, the main products processed by the mini-dairies and cottages are pasteurised milk (45%) and fermented products (yoghurt 27% and fermented milk 22%). Compared to large-scale processing companies, mini dairies, and cottages have limited resources and technology to process high-tech products (Yilma and Tadesse, 2018) such as milk powder, ice cream, ghee, and butter. Similarly, previous studies in the Kenyan dairy industry showed that mini-

dairies are more involved with the processing of pasteurised fresh milk (99.1%) and fermented milk (88%) which use simple equipment and technologies (Odero-wanga et al., 2009).

Influence of COVID-19 containment measures on the supply chain of milk and milk products

Before COVID-19, the quantity of milk received by majority of the processing companies (57%) was less or equal to 4000 litres of milk. The number of companies processing less than 4000 litres increased to 20 (77%) during COVID-19 control measures. This indicates that before COVID-19, there were few companies processing ≤ 4000 litres of milk which increased by 20% during the pandemic. Likewise, companies processing between 4000 and 8000 litres decreased as they received less amount of milk and consequently were downgraded to those receiving ≤ 4000 litres (Table 2). This shows that during the pandemic, companies received less amount of milk than before COVID-19 measures. This could be due to low production of milk during the pandemic or disruption of the supply chains. Although farmers could milk more milk, restrictions could have barred them to sell

Table 3. Quantity of milk received in the processing plant before and during the COVID-19 pandemic outbreak.

Processors	Quantity of milk before COVID-19 (litres)	Quantity of milk during COVID-19 (litres)	Change (%)	P-value
1	1000	400	-60	0.173
2	1600	700	-56	0.153
3	3600	200	-39	0.004
4	6800	6800	0	1.000
5	2000	500	-75	0.003
6	2000	600	-70	0.094
7	6000	4150	-31	0.166
8	1000	500	-50	0.286
9	700	200	-71	0.163
10	5000	1600	-68	0.074
11	1000	500	-50	0.102
12	3500	1300	-63	0.099
13	70	20	-71	0.038
14	4000	4000	0	1.000
15	7000	4500	-36	0.102
16	3400	2833	-17	0.324
17	4300	4300	0	1.000
18	1000	800	-20	0.737
19	1000	600	-40	0.005
20	3000	2000	-33	0.163
21	1000	800	-20	0.678
22	300	200	-33	0.054
23	3500	1100	-69	0.014
24	250	80	-68	0.234
25	1800	900	-50	0.009
26	700	600	-14	0.489
Overall mean	2520	1545	-42%	0.036

Source: Authors

their milk. Moreover, companies could fail to timely collect the milk from collection centres which results in spoilage of milk. For companies that depended on individual farmer supplies could have suffered the most due to restriction of movement during the pandemic as among the disease containment measures.

In addition, as a result of the government's order that people stay at home to curb the spread of the virus, milk processors in Pakistan (Hussain et al., 2020) complained of low milk supply received in the companies and India (Biswal et al., 2020), truck drivers were reported to dump milk as it spoiled due to the delay of milk delivery to processing plants.

Similarly, this occurred in USA (Wang et al., 2020; Wolf et al., 2021) and Nepal (Poudel et al., 2020). Additionally, in Bangladesh, about 27000 tons of milk was spoiled on a daily basis (Ahmed et al., 2021). Contrary to that, milk processors in Kirène factory, Senegal, complained of receiving excess quantity of milk resulting from milk farmers being unable to sell their milk in neighbouring

markets, where consumers purchase raw milk. Whereas in "Fatick" factory, suspension of milk collection and processing was adopted due to traffic bans (Vall et al., 2021).

As for the quantity of milk and the number of distributors of milk (Table 3), in overall there was a significant difference ($P < 0.05$) in the average quantity of milk (litres) supplied among the companies before and during COVID-19. On average, the mini-dairies were receiving 2520 Litres/day before COVID-19, which decreased to 1545 litres/day during COVID-19. Therefore, in general, the mean quantity of milk decreased by 42%.

Furthermore, according to (Ellison and Kalaitzandonakes, 2020) there was limited processing capacity and cold storage resulting in difficulty in processing the raw milk into other products such as cheese. Similarly in Latvia, since there was an increase in the price of raw milk, there was a reduced quantity of milk being processed by the processing companies (Pilvere et al., 2021).

Table 4. Labour availability before and during COVID-19.

Processor	Number of employees before COVID-19	Number of employees during COVID-19	Change (%)	Chi-square test
1	12	9	-25	$\chi^2= 37.7655$ Df= 25 p-value= 0.04
2	5	3	-40	
3	7	4	-42	
4	11	9	-18	
5	5	3	-40	
6	5	4	-20	
7	14	11	-21	
8	5	3	-40	
9	14	10	-29	
10	7	7	0	
11	6	4	-33	
12	5	5	0	
13	12	10	-17	
14	7	4	-42	
15	12	9	-25	
16	7	5	-29	
17	6	3	-50	
18	15	15	0	
19	13	10	-23	
20	3	3	0	
21	7	4	-42	
22	5	3	-40	
23	2	2	0	
24	7	5	-29	
25	7	4	-42	
26	11	8	-27	
Total	210	157	-25	
Overall mean	8	6		

Source: Authors

The management of the flow of goods and services including all processes that transform raw materials into final products, distribution, and transportation serves as the last stage to reach final products hence forward movements (Chitrakar et al., 2021). Hence, the capacity of milk supply to the company could directly reflect on the quantity of milk distributed.

Influence of COVID-19 containment measures on the number of employees and distributors of milk and milk products

The study revealed that on average, there were eight and six employees working in the processing company before and during COVID-19 respectively (Table 4) with a significant difference ($P < 0.05$) in the number of employees before and during COVID-19. This indicated a 25% negative decrease. Whereas as for, the number of distributors during COVID-19 the mean number of

distributors decreased by -40% from an average of 5 to 3 distributors. On the contrary, there was no significant difference ($P > 0.05$) in the number of distributors before and after the COVID-19 pandemic.

During the COVID-19 pandemic, the agriculture sector was regarded as an essential service to both the world's and national economies because it serves as a prime factor to reduce food security issues. Food sectors had to implement non-pharmaceutical measures to ensure they safeguard the health of all personnel. The food processing sector was spotted as a hotspot due to enclosed spaces for working during processing. Dairy products are one example of a labour-intensive food that needs to be prepared swiftly to avoid spoilage before it gets to its final destination or the processing facility. According to Aday and Aday (2020), processing was reduced, suspended, or temporarily discontinued in many factories as a result of the employees who were confirmed to be COVID-19 positive and who were reluctant to report to work because they believed they

Table 5. Number of distributors before and during the COVID-19.

Processor	Number of distributors before COVID-19	Number of distributors during COVID-19	Change (%)	Chi-square test
1	4	1	-75	χ ² calculated= 7.77 df= 18 p-value= 0.98 χ ² tabulated= 28.87
2	3	1	-67	
3	2	1	-50	
6	3	2	-33	
7	6	4	-33	
9	3	2	-33	
11	10	5	-50	
13	4	2	-50	
14	37	28	-24	
16	10	9	-10	
17	6	0	-100	
18	2	2	0	
19	5	2	-40	
20	5	2	-60	
21	5	2	-60	
22	6	2	-67	
25	4	3	-25	
26	6	4	-33	
Total	121	73	-40	
Overall mean	5	3		

Source: Authors

would become ill at work. On the other hand, the difficulty in maintaining social distance within the processing areas, where workers stand side by side, as well as the loud talking or shouting due to noisy environments, which results in the release of air droplets, are just a few of the reasons (Stewart et al., 2020), that food processing plants serve as potential hot spots for such outbreaks. For COVID-19, this is the primary transmission path. Additionally, workers may use public transit to travel to their places of employment, and the majority make low salaries to afford insurance costs. For example, the USA and Canada depend on Mexican workers for their agriculture industries, covering one-third of such jobs. More than 60,000 Mexican seasonal workers enter Canada for agro-industries. The cross-border restriction of Mexican labours to the USA and Canada affected the dairy processing industries (Richards and Rickard, 2020). The Labour shortage was also seen in India (Yadav and Agarwal, 2021), Bangladesh (Zabir et al., 2021), and Japan (Zhang, 2021) due to travel restrictions. According to Luckstead et al. (2021), the rapid spread of the COVID-19 disease laid off many infected workers, resulting in difficulties with reduced production and processing capacity and several dairy plants closing down, disrupting the food supply chain, despite efforts to socially isolate the necessary agricultural workers, such as those in the dairy sub-sector. In regards to the number of distributors (Table 5), there was no significance

difference ($p > 0.05$) in the number of distributors before and during COVID-19 pandemic outbreak. This indicates that there was no significant drop in the number of distributors working throughout the COVID-19 outbreak and implemented measures to curb the spread of the disease since agricultural product transportation was regarded as an essential service. This was in contrast to a research conducted in France, which indicated a 60% decrease in the usage of trucks for food distribution during COVID-19 restrictions (Bakalis et al., 2020; Mouloudj et al., 2020).

Moreover, studies in USA and China observed that the dairy industry was heavily affected by disruption and difficulties in moving milk within the supply chain (Wang et al., 2020). This resulted in discarding the milk or spoilage hence losses. In Canada, processing facilities were closed down due to low supply of milk thus milk farmers were forced to dump their fresh milk whereas in Bangladesh, the country suffered a loss of approximately sixty-seven million US dollars due to discarding of milk (Weersink et al., 2020; Rahman et al., 2022; Begum et al., 2020).

View on cost of processing in the processing company

Processing cost could be defined as the costs a company

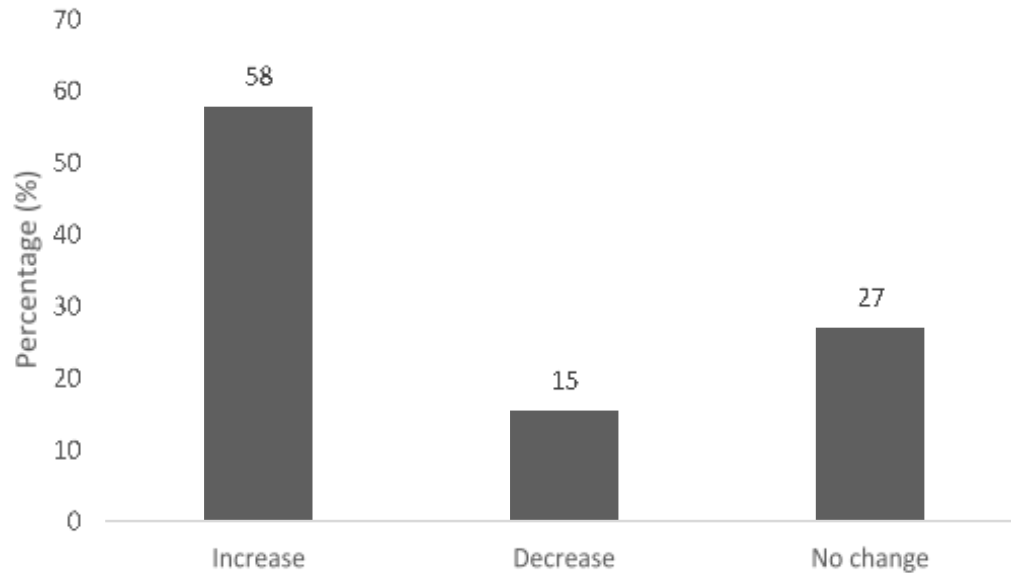


Figure 3. Cost of processing milk in the processing plants.

Source: Authors.

incurs from manufacturing a product or providing services that generate revenue for the company (Odera- Waitituh, 2017). For an effective supply chain, the processing cost should not exceed profits hence generating revenue thus a company may stay in the market longer (Nkwabi, 2019).

In this study, (Figure 3) most processors saw an increase in processing costs 15 (58%) whereas 7(27%) experienced no change in processing cost while four reported a drop in processing cost. Dairy and dairy products are labour-intensive products that require time sensitivity to prevent spoilage. Due to the measures put in place to curb the spread of the coronavirus led to changes in the purchasing behaviour of dairy consumers in Romania (Bruma et al., 2021). This, therefore, resulted in low demand for processed milk, thus reducing profits and losses increasing. In another instance, as reported by (Chandel et al., 2020) the managing director of Dolha Dairy attributed a 40% fall in the sales of processed milk due to reduced purchases by consumers. This was similarly seen in Louisiana, where Briggs et al. (2022) reported an increase in the cost of processing during COVID-19 due to the implementation of safety protocols with the aim of safeguarding the health of employees through the need of installation of washing facilities with running water, and purchasing of personal protective equipment's (masks, gloves, etc.) so as to continue with the day's processing activities. According to Bhandari and Ravishankar (2020), unlike public dairy processors in India, private dairy processors had difficulties obtaining and transporting milk. As a result of the closure of restaurants, hotels, and schools, milk demand decreased. Meanwhile, although input costs for processing costs did

not significantly rise, transportation costs did. The study also revealed that some processors, 7(27%), experienced no change in the processing cost. This could have been led by the swift action taken by the government, through the Kenya Dairy Board which resulted in the withdrawal of processors paying for license fees. This served as a relief to the processors both small and large scale, as they would channel the funds into continual processing even at such difficult times. Moreover, according to the observations, the amount of milk processed decreased in line with the employees. It may be argued that with fewer employees, processing capacity was likewise decreased, resulting in a steady processing cost. Additionally, there was a general decline in the number of distributors. The companies also started using company trucks for distribution, which cut down on processing costs.

Strategies used to ensure constant supply of milk and distribution of dairy milk products

The dairy processing industry, like all other food processing industries, had an obligation to safeguard the health of everyone involved in the food supply chain, including those who worked as suppliers and distributors as well as employees within the processing facility. To ensure continued processing, the companies had to devise the ways they receive milk and distribute the products even during the lockdowns. Although movements were allowed during the day, during the evening there was a complete lockdown. This could have limited milk consumption as eating out of homes and

Table 6. Strategies used to ensure constant supply of milk and distribution of dairy milk products.

Supply strategy	Frequency (n)	Percentage
Independent farmers	6	23.1
Company collection centres	8	30.8
Company agents	9	34.6
Company farms	3	11.5
Distribution strategy		
Use of company vehicles	15	57.7
Engaged private distributors	2	7.7
Local sales	9	34.6

Source: Authors

other social gatherings that could attract the use of milk and milk products were not possible. Therefore, strategies were therefore developed in order to distribute the processed products to customers or larger dairy processors as well as receive their raw materials in the company.

The mini-dairies and cottages assessed in this study (Table 6), used company agents (34.6%) and company collection centres (30%) to ensure that there is a continuous supply of raw milk. Moreover, the majority of companies used company vehicles (57.7%) to ensure the continued distribution of the products. In addition, local sales (34.6%) became an important channel of product distribution. In India, according to Hambarzumyan and Gevorgyan (2021), most processing milk companies adopted new strategies to deliver their processed products to their customers owing to the opportunities coming from prospects of online sale of milk and dairy products. This brought about the deliverance of services to customers' apartments. In Romania, milk was purchased directly from the processors as a result of changes in the purchasing behaviour of dairy consumers (Bruma et al., 2021). Kirene factory in Senegal, received milk quantities in exceeded the company's capacity. This led to the decision to implement delivery quotas as a new strategy to control the flow of milk supplies. In order to understand the behaviour of dairy consumers in short food supply chains, Bruma et al. (2021) performed a study in Romania. They found that the COVID-19 pandemic significantly changed the way that consumers bought dairy products directly from processors. Therefore, we may argue that buying processed milk directly from the processors could result in a similar situation, which would justify the limited volume of local sales.

For that reason, small-scale dairy processors need to improve their mitigation strategies to reduce the effects of future pandemics measures on their supply and distribution. Firstly, contingency planning is essential to mitigate the impact of future pandemics. Small-scale

dairy processors should develop comprehensive contingency plans that include alternative production and distribution channels, workforce management strategies, and communication protocols. A study by Pettit et al. (2013) emphasizes the significance of proactive planning and flexibility in mitigating supply chain disruptions during crises.

Secondly, implementing technology solutions can significantly enhance supply chain visibility and efficiency. This can involve adopting inventory management software, data analytics tools, and automation systems. According to research by Awino and Mutua (2014), leveraging technology can improve supply chain responsiveness and enable better decision-making. By monitoring supply chain activities in real-time and leveraging data-driven insights, small-scale dairy processors can proactively address potential bottlenecks and disruptions.

Additionally, establishing collaborative networks and partnerships within the industry can enhance resilience. Research by Kumar et al. (2018) highlights the importance of collaborative relationships in managing supply chain risks. Small-scale dairy processors can collaborate with other processors, suppliers, and distributors to share resources, information, and best practices. By working together, they can collectively address challenges and find innovative solutions to minimize the effects of future pandemic measures on milk supply and distribution.

Finally, small-scale dairy processors need to adopt digital solutions such as online ordering and payment systems to reduce the need for physical contact and minimize the risk of transmission of COVID-19 and maintaining open communication with customers is vital (Xames et al., 2022). Regularly updating customers about operational changes, product availability, and safety measures through various communication channels, including websites, social media platforms, and newsletters, can help build trust and loyalty. Engaging with customers directly, addressing concerns, and incorporating feedback can strengthen relationships and enhance customer satisfaction.

Policy implications

The impacts of COVID-19 measures on the supply and distribution of milk on small-scale dairy processors have been significant. While the research sheds light on the effects of COVID-19 on the dairy industry and several policies were put in place to support the dairy industry, there are still gaps between the research findings and current policies and practices. The following are the policy implications of the study:

1. One of the major policy implications is the need to enhance the resilience of the dairy industry to future disruptions. The pandemic has highlighted the

importance of resilient and adaptive supply chains to minimize the impacts of disruptions. Policymakers should develop policies that promote the adoption of modern technologies and practices in the dairy industry to enhance resilience.

2. Filling the Gap in Technical Assistance: The study found that small-scale dairy processors face challenges in adopting new technologies and practices to improve their efficiency. However, there is a gap in technical assistance to help these processors overcome these challenges. Governments should fill this gap by providing technical assistance to small-scale dairy processors to help them adopt new technologies and practices. This will improve their efficiency and competitiveness in the market.

3. Bridging the Gap in Cold Storage and Transportation Infrastructure: Governments should work to bridge this gap by investing in cold storage facilities and transportation infrastructure to ensure that milk is transported and stored at optimal conditions. This will help reduce wastage and improve the quality of milk.

4. There is a need to form multi-sectoral cooperation in the implementation of policies to increase the implementation efficiency. This will ensure that, policies formulated are translated to fit the specific sectors hence effective implementations.

In conclusion, the impacts of COVID-19 measures on the supply and distribution of milk on small-scale dairy processors require policymakers to take proactive steps to address the challenges facing the industry. By doing so, policymakers can help small-scale dairy processors to weather the pandemic's economic impacts and contribute to building a more resilient and sustainable dairy industry.

Conclusion

The COVID-19 pandemic has had significant impacts on the supply and distribution of milk on small-scale dairy processors. This study has shown that the pandemic's effects have led to decreased milk supply to the small processing companies, reduced number of employees and increased cost of processing. The study also highlights the challenges faced by small-scale dairy processors in maintaining the milk supply and distribution chains and the adoption or modification of new technologies. Therefore, the study has identified potential policy recommendations and improvements that could support small-scale dairy processors in maintaining their businesses during pandemics as well as allow formulation and implementation of policies that allow for flexibilities that can be tightened and eased to ensure resilience of the small processors.

Future research scope

There is still room for additional research in this area,

despite the fact that this study has given useful insights into the effects of COVID-19 measures on small-scale dairy processors. Future research may focus on a number of areas, including:

Comparative Study: The effects of COVID-19 measures on both large- and small-scale dairy processors could be evaluated through a comparative study. This would make it easier to spot similarities and differences in the challenges faced by small-scale dairy processors and possible legislative solutions.

Analysis of Consumer Behaviour: Future studies could examine how consumer behaviour changed in response to the COVID-19 measures, particularly in relation to how much milk and milk products consumers consumed. This would make it easier to pinpoint the variables that affect consumer behaviour and possible effects for small-scale dairy processors.

Sustainable practices: Future research could also focus on promoting sustainable practices in small-scale dairy processing, taking into account the impacts of COVID-19 measures. This would help to improve the sustainability of the dairy sector and support the long-term viability of small-scale dairy processors.

Lastly, research can also be conducted to explore the potential of technology adoption in mitigating the impacts of COVID-19 on small-scale dairy processors. This includes the adoption of digital platforms for marketing and sales, as well as the use of technology to improve efficiency in milk production and processing.

Overall, future research in this area will be critical in identifying and addressing the challenges faced by small-scale dairy processors during and after the pandemic.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Compliance with food safety standards by beef vendors at butcheries in Kamuli district, Uganda

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Butcheries are the leading retailers of beef in Uganda and their level of compliance with food safety standards is unknown. The aim of this study was to determine the compliance by beef vendors in Kamuli district with the US 736:2019 standard for hygienic requirements for butcheries. A survey questionnaire and observation checklist on sanitation, hygiene, and beef handling were used to collect data from 60 butcheries. More than 75% of beef vendors complied with the inspection, storage, and some sanitation and hygiene requirements, however, many violated the transportation and construction requirements. Self-reported surveys revealed that 96.7% of vendors sold inspected beef, 83.3% of butchery facilities were inspected at least once a month and all vendors stored beef for less than 36 hours. Beef vendors (76.7%) reported washing beef handling tools with water and soap whereas 96.7% cleaned butcheries every day. Sixty percent (60%) of vendors transported beef using motorcycles, and 23.3% used tricycles. All beef vendors observed had short hair, short fingernails, and did not wear jewelry. Only 15% of vendors wore protective clothing when handling beef. Butcheries had wooden walls (71.7%), and their floors were either wooden or bare ground (65%). This study demonstrated a need for food safety interventions to emphasize sanitation and personal hygiene practices, safe transportation of beef, and the hygienic construction of butchery facilities.

Key words: Beef butcheries, compliance, food safety, beef handling.

INTRODUCTION

Beef is the number one meat consumed in Uganda and Kamuli district (Agriterria, 2012; Ikendi, 2019). The demand

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for beef in Uganda is likely to increase due to the rapidly growing population estimated at an annual growth rate of 3.3% (World Population Review, 2022). Roadside and market stall butcheries are the main retailers of raw beef to consumers at both household level and retail food establishments, contributing 75 to 80% of sales in the industry (Agriterria, 2012). Despite its high nutritional value, beef in Uganda has been reported to be contaminated with pathogenic microorganisms (Bogere and Baluka, 2014; Lujjimbirwa et al., 2022). Recently beef has been implicated in gastrointestinal anthrax outbreaks in Uganda (Nakanwagi et al., 2020; Musewa et al., 2022). Beef vendors at butcheries must protect raw beef from contamination before it is sold to the final consumer for further handling, processing, and cooking.

In order to protect public health, the Uganda National Bureau of Standards (UNBS) and the Ministry of Agriculture Animal Industry and Fisheries (MAAIF) have provided hygiene requirements for butcheries in the standard US 736:2019, released in 2019 (UNBS, 2019). This standard provides requirements before opening a butchery, requirements for construction, equipment, operation, personal hygiene, storage of meat, inspection of meat and butcheries, and requirements for transportation of meat. The standard requires personnel operating butcheries to be trained in food and personal hygiene practices before starting to work and to understand the hygiene and safety requirements. Complying with food safety requirements is important for protecting the public from foodborne illnesses. Compliance is in part fostered through using a collaborative approach when conducting inspections, whereby food business operators form partnerships with inspectors and work with them to learn about best practices and solve food safety issues at their facilities (Buckley, 2015). Additionally, compliance can be encouraged by designing educational interventions that identify and address barriers experienced by food business operators (Chen et al., 2021).

According to the population census of 2014, 18.6% of the populations in Kamuli district were children under the age of four years (UBOS, 2017). Children in this age group, especially after six months are susceptible to foodborne illnesses, since their immune systems are not yet fully developed (CDC, 2022) to fight against pathogens that may be introduced through ingesting foods other than breast milk. Diarrhoea is one of the common signs of foodborne illness and can lead to dehydration and death among children below five years (Talbert et al., 2019). Busoga region, where Kamuli district is located, had the second highest prevalence of diarrhoea according to the Uganda Demographic and Health Survey of 2016 (UBOS and ICF, 2018). Nabongo et al. (2014) also reported that diarrhoea is the third leading cause of death among children below five years in this region.

Improper handling of highly perishable foods such as

beef can lead to cross contamination of ready-to-eat foods and food contact surfaces and can contribute to these diarrhoea related diseases and death. Although in Uganda beef is typically cooked before consumption (Asada, 2019), it is important that vendors sell raw beef with the lowest microbial load possible to minimize subsequent contamination with other food contact surfaces during preparation by consumers at home. The aim of this study was to determine if beef vendors in Kamuli district follow the hygienic requirements set out in Uganda Standards for butcheries (US 736:2019) and to identify services available to support vendors to meet the requirements.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Kamuli district in Uganda from October to December of 2021, following approval from Iowa State University's Institutional Review Board (IRB ID - 21-125), and the Makerere University School of Social Sciences Research Ethics Committee (MUSSS-2021-87).

Survey questionnaire development

A survey questionnaire and observation checklist was used to collect data in this study. The survey questionnaire and observation checklist designed to reflect the minimum hygiene requirements for butcheries in the standard US 736:2019, included both multiple choice questions and open-ended questions, and adopted and modified questions from previous studies by Mirembe et al. (2015) and Chepkemoi et al. (2015). A draft of the survey questionnaire and observation checklist was evaluated for content validity by six food safety experts at Iowa State University and Makerere University. Experts evaluated whether the different aspects of the standard US 736:2019 were represented in the questionnaire and observation checklist and provided feedback on the organization of the different sections in the questionnaire. A revised questionnaire and observation checklist were piloted with six beef butcheries in Butansi sub-county in Kamuli district to verify that content was easy to understand and to determine if both the face-to-face interviews and observations could be completed within one hour or less. Vendors who participated in the pilot test were not recruited in the research study and their data was not used in generating the results presented in this paper. The final survey questionnaire had a total of 29 questions, categorized into five sections which were butchery operation-related questions (10), sanitation (five questions), personal hygiene (four questions), storage practices (two questions), and demographics (eight questions). The observation checklist covered construction, hygiene, sanitation of personnel and surroundings at the butchery.

Participants and inclusion criteria

Researchers contacted personnel at the Kamuli district's Veterinary and Public Health departments, and two people (one from each department) consented to participate in the study. These individuals are involved in ensuring that beef sold at butcheries is safe for human consumption.

Sixty-two vendors at beef butcheries operating at the time of this study were contacted, and 60 (96.8%) were allowed to participate in the study. These vendors' butcheries were located in Kamuli

Municipal Council, and in five out of 12 rural sub-counties which included Namasagali, Balawoli, Namwendwa, Bugulumbya and Nawanyago. The rural sub-counties and municipal council were from all three counties (Bugabula County North, Bugabula County South, and Buzaaya County) of Kamuli district.

Only adults of 18 years and above who sold raw beef in Kamuli district were allowed to participate in the study. Participants were given a consent form with information about the study or verbally given the same information in Lusoga language (for vendors), and only those who agreed to participate were interviewed. According to the IRB requirements, participants were informed about their freedom to stop participation at any time and to skip any questions they did not want to answer.

Survey questionnaire administration

For beef vendors, the survey questionnaire and observation checklist were administered on the same day and by the same researcher to ensure the consistency of administration of the survey tool. Face-to-face interviews were conducted in the local Lusoga language to help minimize misinterpretation of questions and to allow respondents who were unable to read or write in English to participate in the study. Interviews were conducted on the butchery premises to allow for observations to be taken with permission from the respondent.

In-depth interviews with personnel from the Veterinary and Public Health departments of Kamuli district were conducted on their office premises and guided by pre-developed questions about services available to assist beef vendors to comply with food safety requirements. In-depth interviews were conducted in English and audio-recorded with permission from the respondent.

Data analysis

Data from surveys and observation checklists were entered into Microsoft Excel and exported to JMP Pro, version 16 statistical software for analysis. Descriptive statistics of percentages and frequencies were calculated. Responses to open-ended questions were content-analyzed for common themes. Vendors' beef handling practices were compared to the requirements in the standard US 736:2019, and any gaps where vendors did not comply with the standard were identified. Audio-recorded in-depth interviews were transcribed into Microsoft Word, and information about services available to assist beef vendors in complying with food safety requirements was color-coded in Microsoft Word and extracted. It should be noted that the limited number of food safety personnel ($n = 2$) who participated and the lack of representation from the Ministry of Agriculture, Animal Industry and Fisheries may negatively impact some of the results discussed in this paper.

RESULTS AND DISCUSSION

Supportive services to beef vendors in complying with requirements.

The in-depth interviews showed that the Department of Public Health and the Veterinary Department of the Kamuli district work together to provide various services to support beef vendors within the district and to ensure that beef sold to the general public is safe for human consumption. Table 1 shows the services provided to beef vendors.

The services provided either directly or indirectly promote beef vendors' compliance with food safety requirements of the standard US 736:2019. Although a number of services were provided, personnel participating in in-depth interviews noted that hygienic packaging and transportation of beef from the slaughterhouse to the butchery was still a challenge. Personnel also added that slaughter facilities were not enough during festive seasons, so some animals were slaughtered in open fields, exposing beef carcasses to environmental contamination.

Government inspection of small food facilities is key in ensuring compliance with food safety standards, as it gives inspectors an opportunity to build relationships with facility operators, and learn the challenges experienced while trying to follow requirements and provides a platform for inspectors to share information about best practices to minimize food contamination (Buckley, 2015). Barnes et al. (2022) also added that inspection encourages positive behavioral change both when interacting with business operators and when results from inspections are disclosed to the public. Food safety inspectors are better prepared for their job when they are trained, and their proficiency increases with experience (Barnes et al., 2022). This present study did not inquire about inspectors' training or experience and did not obtain details about the results of butchery inspections.

Demographic characteristics of beef vendors

A total of sixty beef vendors from Kamuli Municipal council, and five rural sub-counties participated in this study. All beef vendors (100%) were male with 71.2% being Muslims. A majority of the beef vendors (40.7%) were between 18 and 30 years of age. The most commonly spoken language (98.3%) by the beef vendors was Lusoga. Almost sixty-four percent (63.8%) had completed primary education and above with highest level being Uganda Certificate of Education (UCE). Beef vendors (49.2%) had experience running a beef butchery business for greater than 10 years. Table 2 shows demographic characteristics of beef vendors in this study.

The beef butchering business in Kamuli district is dominated by men, like other butcheries in other parts of Uganda (Mirembe et al., 2015; Heilmann et al., 2015; Jeffer et al., 2021), Ethiopia (Zerabruk et al., 2019), Nigeria (Alimi et al., 2022), and Ghana (Adzitey et al., 2020). The level of education among beef vendors in this present study is similar to that reported by Zerabruk et al. (2019) and Tegegne and Phygo (2017) among Ethiopian butchers whereby a majority (85 and 52.7%) had studied up to an elementary level. Lamunu et al. (2022) also reported that 50.7% of beef vendors in Wakiso district in Uganda had not studied beyond the primary level. In this present study, the vendors' level of education is lower compared to that reported by Mirembe et al. (2015) and

Table 1. Supportive services to beef vendors in Kamuli district.

Service	Description
Movement permits	These permits are required for inter-district movement of animals and are issued following examination of animals by the veterinary officer. Movement permits contain information about the owner of the animal, the district where the animal is departing from, and the district where the animal is to be taken. This ensures that only healthy animals are transported between districts and minimizes spread of diseases
Issue licenses	Licenses allow vendors to open and operate butcheries. When issuing licenses, beef vendors are informed about the hygiene and health requirements and are told what facilities they must have at their butchery
Slaughter facilities	These facilities include roofed and enclosed slaughterhouses, open slaughter slabs, and open shades. Management provides clean water to use at the slaughter facilities. During festive seasons, temporary locations are designated to meet the additional demand for slaughtering more animals
Inspection of beef at the slaughterhouse	At the slaughterhouse, veterinary officers inspect animals before and after slaughter to ensure that the beef to be distributed will not compromise the health of consumers or that of other animals within the community. Beef that passes postmortem inspection is stamped as a sign to distinguish it from any beef that is not inspected in the market. Beef that does not pass postmortem inspection is condemned and discarded. Personnel must also ensure that beef from the slaughterhouse is packaged and transported with minimal contamination
Inspection of butcheries	Inspection at butcheries involves monitoring the health and hygiene of beef vendors, sanitation of butchery premises, and verifying that beef sold was inspected and stored properly for not more than two days. Inspectors also check whether beef vendors wear protective gear including an overcoat and gumboots or closed-toe shoes. Through inspection, inspectors also respond to calls from the public about potentially suspicious beef at butcheries
Medical forms showing vendors' health status	Beef vendors are tested by health professionals at the district hospital to confirm that they are free from communicable diseases. Beef vendors must have the medical form during inspections, and the form must be renewed every six months. Occasionally, the management at the slaughterhouses demands that beef vendors present their medical forms before they receive any beef
Capacity building	Occasionally, Kamuli district works together with non-government organizations to organize workshops that empower the butchery community. The respondent gave an example that in 2021 Kamuli district co-organized a workshop with the International Livestock Research Institute (ILIRI). This workshop was focused on preventing tapeworms and was attended by several stakeholders including veterinary officers, public health officers, extension agents, and beef handlers from slaughterhouses and butcheries in Kamuli district. Inspectors also remind beef vendors about safe beef handling practices during inspections

Extracted from in-depth interviews with personnel from the Public Health (n = 1) and Veterinary (n = 1) departments.

Kunyanga et al. (2021) in Kampala and Kenya, where 57.5 and 60% of butchers, respectively, had obtained secondary education or higher. The low level of education among beef vendors in Kamuli district could be one of the hindering factors for vendors to comply with the requirements. Food safety educators should consider the simplification and translation of food safety standards to languages understood by vendors. Additionally, pictorial presentation of the standard and culturally appropriate visuals with minimal text about safe beef handling practices can be utilized during training to support compliance by the beef vendors (Nabwiire et al., 2022).

The beef butchering business in Kamuli district is fairly stable, with 49.2% of vendors having been in business for greater than 10 years. Similarly, Kunyanga et al. (2021)

in Kenya, and Alimi et al. (2022) in Nigeria reported that 54%, and 67.6% of meat handlers in their studies had greater than 10 years of experience in the meat butchering business, respectively. Food safety educators should use strategies that encourage all beef vendors to use safe meat handling practices regardless of their level of experience in the butchering business. Additionally, refresher training can be incorporated into food safety training programs to reinforce food safety practices among returning beef vendors (McFarland et al., 2019) and the train-the-trainer model leveraging vendors' experience in using safe practices can be adopted in the future (Warner et al., 2014).

Youth and Muslims dominate the beef butchery business in Kamuli district as in Wakiso district, where

Table 2. Demographic characteristics of beef vendors at butchereries in Kamuli district^z.

Demographic variable	Number of respondents (<i>n</i>)	Percentage of respondents (%)
Gender (<i>n</i> = 60)		
Male	60	100
Female	0	0
Age (<i>n</i> = 59)		
18-30	24	40.7
31-40	20	33.9
41-50	11	18.6
51 and above	4	6.8
Language (<i>n</i> = 59)		
Lusoga	58	98.3
Luganda	1	1.7
English	0	0
Religion (<i>n</i> = 59)		
Muslim	42	71.2
Christian	17	28.8
Level of education (<i>n</i> = 58)		
Did not go to school	3	5.2
Below primary seven	18	31
Primary seven	15	25.9
Some ordinary level classes	10	17.2
Senior four – UCE	12	20.7
Years in butchering business (<i>n</i> = 59)		
Less than 1 year	2	3.4
1.5 to 5 years	17	28.8
6 to 10 years	11	18.6
Greater than 10 years	29	49.2

^zOctober to December, 2021; participants included persons who were 18 years or older involved in selling raw beef in Kamuli district in Uganda.

55.2% of butchers were between 18 and 35 years of age, and 61.2% were Muslims (Lamunu et al., 2022).

Beef vendors' compliance with hygienic requirements for butchereries

In this study, beef vendors complied with some requirements in the US 736:2019 standard while violating others. Table 3 shows the percentage of beef vendors complying with or violating different requirements in the standard.

Meat and meat products sold at butchereries

Eighteen percent (*n* = 11) of vendors sold only beef, whereas the remaining sold beef and meat from other species, including goat (57%, *n* = 34), turkey (1.7%, *n* = 1), and sheep (1.7%, *n* = 1). No vendor sold pork probably because the highest percentage (71.2%) of

vendors identified as Muslim. In addition to selling different meat types, 80% (*n* = 48) of vendors sold offals including bovine or goat intestines, liver, or lungs. Twenty percent of vendors (*n* = 12) also sold heads and legs of goats and cattle at their butchereries. Vendors separated offals from skeletal muscle meat using separate sisal ropes (50%, *n* = 24 of 48), separate metallic hooks (18.8%; *n* = 9), leaving space between meat and offals placed on the same table (14.6%; *n* = 7), using separate tables (10.4%; *n* = 5), displaying meat and offals in different shades (4.2%; *n* = 2), and putting the offals in a separate plastic bucket (2.1%; *n* = 1). In this study, although beef vendors endeavoured to separate offals from muscle meat, the practice of using ropes and spacing meat and offals on the same table as reported at 64.6% of butchereries, may lead to cross-contamination if surfaces are not thoroughly cleaned and sanitized between uses (Fasanmi et al., 2010; Kirchner et al., 2021). The results of this study are similar to those reported by Bogere and Baluka (2014) and Jeffer et al.

Table 3. Percentage of beef vendors (%) complying with or violating different hygiene requirements of the standard (US 736:2019) at butcheries in Kamuli district.

Category used for research purposes	Section in Standard US 736: 2019 (UNBS, 2019)	Number of vendors	Percentage of vendors complying with requirements	Percentage of vendors violating or using inadequate practices
Meat and meat products sold at butcheries	Section 5.3: Meat vendors must construct a separate area dedicated to handling offals.	48 ^a	35.4	64.6
Source of beef carcass and inspection of meat and butcheries	Section 10.3: All meat sold at butcheries must be slaughtered from an approved abattoir.	60	95	13*
	Section 10.1 and 12: All meat sold at butcheries must be inspected and declared fit for human consumption by an authorized meat inspector.	60	96.7	3.3
	Section 12.1: Butcheries must be inspected by an authorized inspector.	60	98.3	1.7
Packaging and transportation of beef	Section 11: Beef vendors must transport meat to butcheries using a clean closed vehicle or container that has smooth surfaces made of non-absorbent material for easy cleaning. The packaging container used must protect meat from flies and dust during transportation	60	16.7	83.3
Displaying meat at butcheries	Section 7.1: Meat at butcheries must be suspended using metallic hooks that can be washed without corrosion.	60	51.7	76.7*
Storage of meat at butcheries	Section 9: In absence of cold storage services, meat can be stored by hanging to allow aeration around it for a maximum of 36 hours.	59	98.3	5.1*
Cleaning meat handling tools	Section 6: Vendors must clean all tools used for handling meat at butcheries.	60	76.7	23.3
Cleaning of butchery premises	Section 7.5: Vendors must remove waste from butcheries and dispose it of daily.	60	96.7	3.3
Construction materials for floors and walls of butcheries	Sections 5.6: Butchery walls must be constructed with cleanable, and non-absorbent materials.	60	8.3	96.7*
	Sections 5.7: Butchery floors must be constructed with cleanable, and non-absorbent materials.	60	35	65
Flies and methods of controlling them	Sections 7.1 and 5.11: Beef vendors must protect meat from flies and must use fly-proof screens at their butcheries.	60	20	80
Personal hygiene	Section 8.11: Beef vendors must wash hands regularly with clean water and soap, and dry them using disposable towels	60	54	46
	Sections 8.6, 8.8, 8.7, and 8.13: Beef vendors must not wear jewelry, must have short fingernails, short or covered hair, and must cover injuries on hands if present	60	100	0

Table 3. Contd.

Category used for research purposes	Section in Standard US 736: 2019 (UNBS, 2019)	Number of vendors	Percentage of vendors complying with requirements	Percentage of vendors violating or using inadequate practices
Personal hygiene	Section 8.5: Beef vendors must wear protective clothing	60	15	85
	Sections 8.3 and 8.12: Butcheries must have a separate person to handle money	29	42.4	57.6
	Section 8.9: Vendors must not eat food from inside the butchery	29	54.2	57.6*
	Section 8.12: Vendors must not store personal items inside the butchery	29	56.7	43.3

^a48 is the number of beef vendors selling offals. *Total percentages are greater than 100 because some vendors provided multiple responses, some of which complied with requirements and others did not.

(2021) where butchers mixed beef with offals. The gastrointestinal tract (GIT) of cattle can harbour many microorganisms including pathogenic strains of *Escherichia coli* (Sapountzis et al., 2020). Mixing meat and offals may lead to cross-contamination with foodborne pathogens. Beef handlers at butcherries should ensure meat/beef and offals are separated from each other to prevent the contents of the GIT from spilling onto muscle meat during the display at the butchery and that the materials or surfaces used to separate them are cleaned and sanitized between batches.

Source of beef carcass and inspection of beef and butcherries

Ninety-five percent ($n = 57$) of vendors in this study obtained meat and meat products from slaughterhouses, and 13% ($n = 8$) sold meat slaughtered from tree shades or their backyards. Ninety-six percent ($n = 58$) of vendors reported that they sold beef that had been inspected and stamped before transporting it to their butcherries. Eighty-three percent ($n = 50$) of vendors received additional inspection at their butcherries at least once a month, 15% ($n = 9$) were inspected less than five times a year, and one (1.7%) had never been inspected. From the in-depth interviews, personnel from the Veterinary and Public Health departments confirmed providing inspection services at approved slaughterhouses and butchery premises, which enabled 95% of beef vendors in this study to comply with the inspection requirements. Similar to this study, Kyayesimira et al. (2019) reported 100% compliance with antemortem inspections at approved slaughterhouses in Kampala district but only 25% compliance in Mbale and Mbarara districts in Uganda. The percentage of butcherries inspected once a month (83%) in this study is similar to the 71.6% reported by Lamunu et al. (2022) among butchers in Wakiso district in Uganda. However, this percentage (83%) is several times

higher than the eight percent of butcherries reported by Jeffer et al. (2021) in Kampala district. Similarly, Adzitey et al. (2020) reported that 70% of meat sellers in Tamale Metropolis of Ghana obtained meat from designated abattoirs and 30% sold meat slaughtered from the backyards.

Packaging and transportation of beef

In this study, beef vendors used a variety of ways to package and transport beef from slaughterhouses to their butcherries. Before transportation, 50% ($n = 30$) of vendors packaged beef in woven polypropylene bags, 36.7% ($n = 22$) used wooden boxes, 13.3% ($n = 8$) used cardboard boxes, and 1.7% ($n = 1$) used a plastic bucket. Fourteen beef vendors (23%) reported covering beef with black polythene bags (18.3%), tarpaulin (3.3%), and paper (1.7%). Two vendors (3.3%) tied beef carcasses on tree logs. Twenty-eight (46.7%) of the vendors either hired or shared packaging materials with other butcherries. Ten vendors (17%) reused woven polypropylene bags and polythene bags, washing them between uses and replacing them after two rounds of using them or on a weekly or monthly basis. This practice can result in cross-contamination if the bags are not washed and sanitized properly. To transport beef, 60% ($n = 36$) of vendors used motorcycles, 23.3% ($n = 14$) used tricycles with carrier compartments, 16.7% ($n = 10$) used closed vehicles, seven percent ($n = 4$) used wheelbarrows, 1.7% ($n = 1$) used bicycles, and eight percent ($n = 5$) walked with the beef to their butcherries. Beef vendors (21%, $n = 13$) individually owned the means of transportation whereas 78.3% ($n = 47$) either hired or shared transportation with neighboring butcherries. In order to observe packaging and transportation of beef, a slaughterhouse in Kamuli district was visited during their working hours. It was observed that the wooden box had a metallic lining that had cracks and needed replacing for it to be cleaned

effectively. During transportation from the slaughterhouse to the butchery, beef carcasses in the wooden box and tricycle were left exposed to the environment and could become contaminated. It was also noticed that the wooden box and the tricycle made more than one trip from the slaughterhouse, delivered beef to at least four butcheries in each trip, and were not cleaned between trips which could lead to cross-contamination between batches. Bogere and Baluka (2014) reported that microbial contamination of meat increased from the slaughterhouse to the butchery. Contamination could come from containers that are not cleaned properly, and from dust and flies that may come in contact with the meat that is not covered during transportation. Jeffer et al. (2021) and Kyayesimira et al. (2019) also reported that polypropylene bags and wooden boxes were common packaging materials used and were shared by butchers in Kampala, Mbale, and Mbarara districts. Additionally, Jeffer et al. (2021) and Kyayesimira et al. (2019) reported that meat was transported using motorcycles at 84.2 and 54% of butcheries respectively. The use of motorcycles to transport beef was also reported among butcheries in Meru (59.8%), Kitui (60.8%), Nairobi (31%) and Isiolo (39%) counties in Kenya (Kunyanga et al., 2021; Chepkemai et al., 2015). Kunyanga et al. (2021), however, reported that less than 12% of butcheries in Meru, Kitui, and Tharaka Nithi counties in Kenya used wooden boxes to transport beef.

To reduce contamination risks during packaging and transportation of beef in Kamuli district, beef vendors should be educated on the food safety requirements outlined in the standard and emphasis should be put on cleaning and sanitization schedules for transportation and packaging materials used. Single-use, waterproof plastic linings could be placed inside the wooden box and the lining discarded after use. A lid for the wooden box should be designed and used to cover the box during the transportation of beef carcasses. Cleanable plastic bins with lids can be used as primary packaging for beef carcasses transported on tricycles. Persons in charge should not allow any beef not hygienically packaged to exit the slaughterhouse.

Displaying meat at butcheries

During the day, beef vendors displayed meat by hanging it inside the butchery using woven sisal ropes (70%), or metallic hooks (51.7%) and placing beef on wooden tables (6.7%), three of which were covered with cardboard boxes. One vendor used a rusted metallic hook, whereas 88% ($n = 37$ of 42) used visibly dirty ropes to hang beef. Displaying beef by hanging was also observed at nearly all butcheries in Meru, Kitui, and Tharaka Nithi counties in Kenya (Kunyanga et al., 2021), in Kampala, Mbale, and Mbarara districts in Uganda (Kyayesimira et al., 2019), and in Ethiopia (Zerabruk et

al., 2019). In contrast to this study's results, in Ghana, 48% of meat sellers displayed meat by placing it on open tables (Adzitey et al., 2020). In this present study, sisal ropes, which are rough and difficult to clean, were the most common item used to hang beef and other meat products. The practice of using ropes to hang/display beef at the butchery should be avoided since they cannot be cleaned properly and can lead to contamination of the beef. Non-degrading metallic hooks as recommended by the standard should be encouraged for displaying beef at butcheries, as they may be cleaned more effectively. Vendors should also be informed about the tenderization benefits of hanging raw beef as opposed to placing it on tables (Ramanathan et al., 2020).

Storage of beef at butcheries

Storage conditions and storage time are critical when handling raw meat. Only 10.2% ($n = 6$) of beef vendors in this study reported selling out all beef on the same day it is brought to the butchery. Eighty-eight percent ($n = 53$) of beef vendors occasionally took up to two days (from the morning of the first day to the afternoon of the second day) to sell all the beef. When beef remains at the end of the first day, 67.9% ($n = 36$) of beef vendors indicated leaving the beef hanging inside the butchery overnight, 26.4% ($n = 14$) stored it in a refrigerator, 5.7% ($n = 3$) covered it in a cardboard box inside the butchery, and 3.8% ($n = 2$) either gave it out or took it home to feed their family. Although all beef vendors complied with the storage requirement because they had beef at the butchery for less than 36 hours and hung it during storage, the beef can accumulate foodborne pathogens (Bogere and Baluka, 2014) which can cause consumers foodborne illnesses. In this study, the percentage of vendors holding meat at their butchery for more than one day was more than twice the 34.2% reported by Jeffer et al. (2021) among butcheries in Kampala district. Kampala district ($N = 1,507,080$) has three times the population of Kamuli district ($N = 486,319$) (UBOS, 2016) and so beef vendors in Kampala can sell higher quantities of beef within the same amount of time since the demand is higher. Jeffer et al. (2021) and Mirembe et al. (2015) also report that 78 and 65.8% of butchers respectively stored leftover beef by hanging it inside their butcheries. Hanging meat is used as a storage method at 82.8 and 46.5% of butcheries in Nairobi and Isiolo counties in Kenya (Chepkemai et al., 2015) respectively. On the contrary, all butcheries in Kasama district in Zambia used deep freezers or walk-in cold rooms to store meat until it was all sold (Hanyinza et al., 2020). Adzitey et al. (2020) also reported that all meat sellers in Tamale Metropolis of Ghana stored leftover meat in a refrigerator. Beef vendors in Kamuli district should be encouraged to use cold storage or discouraged from stocking more beef than they can sell the same day it is brought to the

butchery. Vendors should also consider increasing marketing efforts and identifying restaurants and/or businesses that can purchase beef for cooking if stock remains after a day of storage.

Although cold storage is the appropriate form of storing raw meat (FDA, 2018), as it reduces the rate of microbial growth, its availability continues to be low, at less than 40% of butcheries in Uganda (Jeffer et al., 2021; Kyayesimira et al., 2019), Ethiopia (Zerabruk et al., 2019), and Kenya (Chepkemoui et al., 2015). Five beef vendors reported that their refrigerators stored beef alone, whereas two mentioned that they created space between beef and other items in the refrigerators. Storage of raw beef with other food items in the same refrigerator was also reported by Mirembe et al. (2015) among butcheries in Kampala. Beef vendors in Uganda are allowed to display and store meat by hanging it inside butcheries which are located in a warm tropical climate with temperatures ranging from 64°F (18°C) to 86°F (30°C), and humidity ranging from 66 to 85% (World Weather, 2023).

Keeping beef at such temperatures for 36 hours allows multiplication of microorganisms already present on the beef. In fact, Bogere and Baluka (2014) reported that meat samples collected from butcheries in Kampala were contaminated with *Staphylococcus aureus* (10^5 CFU/g), *E. coli* (10^5 CFU/g), and coliforms (10^6 CFU/g), all of which were above the maximum allowable limits for beef carcasses and cuts. Although meat in Uganda is typically thoroughly cooked before consumption (Asada, 2019), pathogenic microorganisms such as *Staphylococcus aureus* (10^6 CFU/g) can produce toxins that are not destroyed by heat (Regenthal et al., 2017). The 36-hour allowance in the US 736: 2019 standard to hang/hold raw meat at butcheries in the absence of cold storage should be reduced to 10 hours following slaughter to protect consumers from potential bacterial toxins. This recommendation is based on the findings that Staphylococcal toxins are detectable after 10 hours of exposing raw beef to warm temperatures of 37°C, or after 48 hours of exposure to 20°C temperatures (Grispoldi et al., 2019).

Cleaning beef handling tools

Seventy-six percent ($n = 46$) of beef vendors used water and soap to clean knives and pangas used for chopping beef at their butcheries, whereas 23.3% ($n = 14$) did not use soap during cleaning. Additional materials used for cleaning were sand (53.3%, $n = 32$), a piece of woven polypropylene bag (38.3%, $n = 23$), and steel wire (steel wool) (3.3%, $n = 2$). Twenty-six percent ($n = 16$) of beef vendors reported sharpening tools using a filer or a stone, and 23.3% ($n = 14$) wiping tools with reusable towels after washing them or between serving customers. The cleaning methods used by vendors in this study were

also reported by other authors. Kyayesimira et al. (2019) reported that 50% of meat handlers used cold water without soap, 27% used cold water with soap, 17% used hot water and soap, and 6% used sand to clean butchering tools in Kampala, Mbale, and Mbarara districts in Uganda. The percentage of butcheries using water and soap to clean utensils in the present study is much higher than that reported by Chepkemoui et al. (2015) among butcheries in Nairobi (30%) and Isiolo (7%) counties in Kenya. However, the percentage of butcheries using reusable cloths in this present study is much lower than that reported among butcheries in Nairobi (33.8%) and Isiolo (59.9%) counties in Kenya (Chepkemoui et al., 2015).

Although the US 736: 2019 standard requires cleanliness, it does not specify what materials can or cannot be used to clean tools used for handling meat. Some cleaning practices used by beef vendors may pose physical or biological hazards to consumers. Twenty-three percent of beef vendors did not use soap to clean tools. Soap acts as a surfactant, reducing surface tension so that soil and microorganisms (3.53 log reduction) are more easily removed from food contact surfaces (Marriott et al., 2018; Man and Heacock, 2018). Therefore, cleaning tools without soap as observed in this present study may leave pathogens which could be transferred to beef subsequently sold to the consumers. From the results of this study, it is recommended that the standard discourages the use of reusable towels to wipe tools because if they are not cleaned and sanitized on a regular basis, they can harbor microorganisms (Sukumaran and TR, 2021) and contribute to cross contamination. The use of steel wool to clean tools should also be discouraged as it might leave small steel pieces which when ingested can lead to hospitalizations (Abbaslou and Ahmadipour, 2020). Beef vendors should wash beef handling tools with soap and water and sanitize them regularly whenever they become contaminated and allow them to air-dry rather than recontaminating them with reusable towels.

Cleaning of butchery premises

Ninety-six percent ($n = 58$) of beef vendors reported cleaning their butchery every day, one (1.7%) cleaned it once a month, and the other one (1.7%) twice a month. Cleaning by sweeping was reported at 98.3% ($n = 59$) of butcheries and this was followed by mopping (38%, $n = 23$), replacing cardboard boxes on display tables (11.7%, $n = 7$), painting walls (8.3%, $n = 5$), washing display tables (6.7%, $n = 4$), and wiping tables with damp towels (3.3%, $n = 2$). After sweeping, 85% ($n = 51$) of vendors disposed of trash in a pit, a garden, or a bush that was outside their butchery whereas 15% ($n = 9$) put trash in a covered woven polypropylene bag that was kept outside the butchery to be removed for dumping in a communal

trash collection site. Results obtained are in agreement with those reported by Mirembe et al. (2015) and Konyanga et al. (2021). These authors reported that 75.3 and 100% of butchereries cleaned their facilities daily using sweeping and mopping methods. Although 96% of beef vendors in the present study reported cleaning their butchereries every day, it was observed that only 10% ($n = 6$) of butchereries had clean walls, and 90% ($n = 54$) had visibly dirty walls, which suggested that the cleaning methods used by vendors were not effective at removing stains from walls, or that it was not clear to vendors that butchery walls were among the surfaces that needed to be cleaned regularly. Cleaning and sanitizing of butchery premises including meat contact surfaces, floors, walls and the surrounding should be completed on a daily basis to reduce the accumulation of soil.

Construction materials for floors and walls of butchereries

The butchereries whose floor was constructed out of wood were 41.7% ($n = 25$), whereas 35% ($n = 21$) had cement or concrete floors. Butchereries with bare ground floors accounted for 23.2% ($n = 14$). Butchery walls were made of wood (71.7%, $n = 43$), cement or concrete (5%, $n = 3$), glass (1.7%, $n = 1$), or metal (1.7%, $n = 1$). Eleven butchereries (18.3%) were partially open and located on verandas of buildings, four butchereries (6.7%) were completely in the open without any walls whereas 83.3% lacked clear separation between beef and customers. Additionally, 96.7% of butchereries had a roof to protect beef from rain and 98.3% had sufficient lighting for clear illumination of the butchery. Materials used for the construction of butchereries in this study are different from those reported by Jeffer et al. (2021) and Lamunu et al. (2022). These authors reported that 87 and 56.7% of butchereries in Kampala and in Wakiso districts in Uganda respectively had cleanable smooth surfaces and floors. Oyirwoth (2021) reported that 51.2% of butchereries had bare ground floors whereas 48.8% had concrete floors in Nebbi district in northern Uganda. Wood is likely to absorb water and break down faster when washed with water which may explain why 98.3% of vendors opted for cleaning methods that involved using little to no water. The Public Health and Veterinary Departments of Kamuli district should inspect butchereries and only give or renew licenses for those butchereries with walls, floors, and rooves constructed with cleanable material. Additionally, education and resources should be provided to vendors on where they can purchase materials that are cleanable to utilize in their establishments.

Chopping surfaces at butchereries

The standard US 736: 2019, Section 7.4 requires vendors to cut meat on clean food-grade surfaces and to

clean those surfaces regularly. All butchereries studied had a wooden stump on which beef was chopped, and it was observed that the chopping surface was rough. In addition to the wooden stump, 38.3% ($n = 23$) of vendors chopped beef on the wooden display table which was covered with cardboard boxes. These observations were done during working hours when stumps and tables were being continuously used to chop beef, so it was difficult to ascertain whether vendors cleaned them regularly. Chopping meat on a wooden stump is a common practice in several butchereries in Uganda (Mirembe et al., 2015; Heilmann et al., 2015; Bogere and Baluka, 2014), but it should be used with caution since it can lead to cross-contamination of meat as they can harbor microorganisms such as *E. coli* O157 (Kago, 2015). Wooden chopping surfaces are porous, become rough over time, and accumulate nutrients and bacteria providing a conducive environment for the survival of microorganisms, some of which can produce biofilms that make cleaning with traditional methods of scraping and rinsing challenging and inefficient (Sekoai et al., 2020). The use of cutting saws should be encouraged as these are less likely to be contaminated as compared to wooden chopping surfaces (Kago, 2015).

Flies and methods of controlling them

Fly infestation is a general problem for meat butchereries in Uganda. Heilmann et al. (2015) and Mirembe et al. (2015) indicated that 80% of pork butchereries and 78% of beef butchereries in Kampala had been infested by flies. Flies can carry pathogenic microorganisms such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecium*, and *Staphylococcus aureus* which are linked to human infections and animals (Monyama et al., 2022; Heilmann et al., 2015) and so must not be in contact with food or food contact surfaces. In the present study, flies were observed and present at 80% ($n = 48$) of butchereries. Only one butchery (1.7%) had a fly screen. Beef vendors used different strategies and techniques for controlling flies at their butchereries. Thirty-eight percent ($n = 23$) endeavored to sell fresh beef, 35% ($n = 21$) chased flies as they came, 28.3% ($n = 17$) maintained cleanliness, 5% ($n = 3$) sprayed with insect repellent, 3.3% ($n = 2$) smoked wood or charcoal, 3.3% ($n = 2$) smoked mosquito coils, 1.7% ($n = 1$) smeared beef with cooking oil, and 3.3% ($n = 2$) did not use anything to control flies. From the in-depth interviews, food safety personnel reported that beef vendors did not use the required structures with flyproof screens, as they prevented customers from noticing that beef is available for them to buy. Although beef vendors used several means to control flies, these were not effective because flies were still present and could get in contact with beef. The use of fly screens is not yet adopted by most butchery in Uganda (Bogere and Baluka et al., 2014; Jeffer et al., 2021; Kyayesimira et al., 2019).

Table 4. Percentage of beef vendors (%) washing hands at butcherries in Kamuli district ².

Activity	Never	Rarely	Sometimes	Often	Always
After visiting the latrine	0	0	0	1.7	98.3
Returning from outside the butchery	0	18.3	60	20	1.7
Before touching beef	1.7	26.7	26.7	38.3	6.7
After touching beef	1.7	23.3	25	41.7	8.3
After disposing of garbage	0	1.7	23.3	33.3	41.7

Washing hands at different frequencies before or after performing different activities at the butchery ($n = 60$). ²October and December 2021; participants included persons who were 18 years or older involved in selling raw beef in Kamuli district in Uganda.

Vendors from six of the twelve butcherries that did not have flies at the time the study was conducted indicated that selling fresh beef prevented flies from staying around the butchery. The remaining six butcherries either smoked wood, charcoal, mosquito coils, or maintained cleanliness at the butchery. These strategies should be further investigated to identify affordable methods of keeping flies away from raw meat without reducing customers' ability to see the displayed meat.

Personal hygiene

Ninety-eight percent ($n = 59$) of beef vendors in this study reported always washing their hands after visiting the toilet, and 41.7% ($n = 25$) after disposing of garbage. Table 4 shows the frequency of handwashing before or after performing different activities at the butchery.

More than 50% of vendors either always or often washed their hands after visiting the toilet, after disposing of garbage, and after touching beef. Twenty-eight percent of vendors either rarely or never washed their hands before or after touching beef, which could lead to cross-contamination. All vendors (100%) in this study reported washing their hands using soap and water. Water was contained in either a hand-held jerrycan (98.3%) or a bottle (1.7%). Two vendors used sanitizers after washing their hands. Forty-six percent ($n = 27$) of vendors dried washed hands using reusable towels, overcoats, or handkerchiefs. Beef vendors in this study complied with the requirement to wash their hands regularly with clean water and soap, however, 46% did not dry their hands as required and hence could cross-contaminate hands with microorganisms from the reusable drying materials used (Sukumaran and TR, 2021). Similar to these results, Jeffer et al. (2021) reported that 95% of butchers surveyed in Kampala washed their hands. Kyayesimira et al. (2019) also noted that 97.9% of butchers washed their hands after using the toilet, but only 0.33% of butchers washed their hands before touching beef. Mirembe et al. (2015) reported a lower percentage of beef vendors in Kampala washing their hands after visiting the toilet (41%) and before touching beef (11%). A study conducted

in Kenya, also reported that 40 and 18% of butchers in Nairobi and Isiolo counties, respectively, washed their hands before handling meat (Chepkemoi et al., 2015). In Ethiopia, 40.7% of meat handlers reported washing their hands before and after handling meat (Tegegne and Phyo (2017). In Ghana Tamale Metropolis, Adzitey et al. (2020) reported a much higher percentage (96%) of meat sellers always washing their hands before touching meat. The practice of washing hands after using the toilet and before touching beef seems to have improved over the years among beef vendors in Uganda. This study was conducted in 2021 when there were hand-washing campaigns for reducing the spread of COVID-19, which could have contributed to the high hand-washing frequencies reported. It should be noted that although 45% of vendors reported washing their hands before touching beef, on a few occasions when a customer came to buy beef from the butchery, it was observed that some vendors did not wash their hands before serving them, which was different from what was reported. Visual reminders about handwashing should be posted in butcherries, and handwashing stations should be conveniently designed and positioned to simplify performance of this hygienic practice.

The beef vendors were asked the reasons for washing their hands. Seventy-six percent ($n = 45$) wanted to maintain cleanliness, 25.4% ($n = 15$) wanted to prevent diseases with four vendors mentioning COVID-19 as one of the diseases, 22% ($n = 13$) wanted to remove the smell, fat, and blood of beef from their hands, 16.9% ($n = 10$) wanted to avoid germs, and 3.4% ($n = 2$) wanted to prevent flies. One respondent mentioned that handwashing was good behavior and four vendors indicated that money was dirty. Beef vendors in Kampala were also reported to wash their hands to maintain cleanliness and to remove germs from their hands (Mirembe et al., 2015). From the results, it was revealed that beef vendors understand that hand washing can be used as a method to prevent diseases.

The standard US 736: 2019, Section 7.2, requires that the water used for hand washing at the butcherries is potable, and for butcherries to have access to hot water for cleaning food contact surfaces. Water used to perform

sanitation and hygiene activities at butcherries in this study was collected from boreholes (66.7%, $n = 40$) and/or piped (city/treated) water (36.7%, $n = 22$). Only 13.3% ($n = 8$) of vendors used hot water to clean tools, and four of them got it from nearby restaurants. Eighty-six percent of vendors violated the requirement to clean food contact surfaces with hot water. These results differ from those reported by Mirembe et al. (2015) who noted that the main water source for 91.8% of butcherries in Kampala was piped water. Piped water continues to be limited in several parts of Kamuli district, as 85.6% of households use borehole water for drinking (UBOS, 2017).

All beef vendors (100%) in this study had short hair, did not wear jewelry, and did not have wounds on their hands, 98.3% ($n = 59$) had short fingernails, all of which were in line with requirements. Eighty-five percent ($n = 51$) of vendors, however, violated the requirement to wear personal protective coats. Six of those who did not wear protective coats had hung them inside their butcherries. Only one butcher had a first aid kit to use in case of an injury and the rest indicated that they ran to a nearby clinic or pharmacy in case of injury. Beef butcherries were located in trading centers where other businesses including clinics and pharmacies were also located. These results are consistent with those reported by Mirembe et al. (2015) that 89% of butchers in Kampala had short fingernails and 31.5% wore protective clothing. Kyayesimira et al. (2019) also reported that only 22.2% of butchers in Kampala, Mbale, and Mbarara districts wore protective clothing while working. In Nebbi district, Oyirwoth (2021) reported that 83% of butcher operators did not wear protective coats. The results obtained in this present study are similar to those of butchers in Nairobi and Isiolo counties in Kenya, where more than 70% of them did not wear protective clothing (Chepkemoi et al., 2015). Adzitey et al. (2020) and Tegegne and Phyto (2017) also reported that 58% of meat sellers in Ghana Tamale metropolis and 55% of meat handlers in Jigjiga town in Ethiopia did not wear protective clothing when handling meat respectively. On the contrary, 84% of butchers in Meru, Kitui, and Tharaka Nithi counties in Kenya wore protective clothing when handling meat at the butchery (Kunyanga et al., 2021). Additionally, Alimi et al. (2022) reported that 54% of butchers wore protective clothing, whereas 79% wore jewelry while handling beef at the butchery in Nigeria. Beef vendors in Kamuli district should be educated on the importance of wearing protective clothing and encouraged to wear them when handling beef at their butcherries.

Fifty-four percent ($n = 32$) of vendors in this study reported that they had their meals from inside the butchery whereas 57.6% ($n = 34$) ate outside the butchery. From observations, plates and cups were noted inside of butcherries (21.7%, $n = 13$), and 43.3% ($n = 26$) had personal belongings such as wallets and clothing inside butcherries. Twenty-eight percent ($n = 17$) of beef

vendors reported that their butcherries were run by one person, whereas 71.7% ($n = 43$) were run by two or more people. At the time of this study, it was observed that 56.7% ($n = 34$) of butcherries had only one person present. The practice of having one person to handle money and serve meat to customers is common at more than 67% of butcherries in Uganda (Lamunu et al., 2022; Jeffer et al., 2021; Kyayesimira et al., 2019), at greater than 85% of butcherries in Nairobi and Isiolo counties in Kenya (Chepkemoi et al., 2015), and at 75% of butcher shops in Ethiopia (Zerabruk et al., 2019). Money has been reported to carry spoilage and pathogenic microorganisms (Ejaz et al., 2018); hence there is a risk of contaminating beef if the hands of individual beef vendors are not washed properly between activities. Butchery owners should be required to have at least two people at any time the butchery is operating such that responsibilities of cutting beef and handling money are divided, to reduce the risk of contamination of beef.

Beef handlers' knowledge of food safety standards

In this study, 96.6% ($n = 57$) of beef vendors had received some form of training about handling meat. Seventy-six percent ($n = 45$) had been trained by veterinarians or health assistants from the sub-county, district, or town council offices in Kamuli district. Eight percent ($n = 5$) had learned beef handling from their relatives and five percent ($n = 3$) had learned from fellow butchers or their supervisors at work. From the in-depth interviews, food safety personnel also confirmed that health assistants, inspectors, and veterinary officers trained beef handlers and emphasized the meat handling requirements when issuing them licenses, when conducting routine inspections at butcherries, and during occasional meetings held at slaughterhouses and seminars organized by the district, sub-county, or town council. These results are consistent with those obtained from a study conducted in Kampala by Jeffer et al. (2021) where 94% of butchers reported that they had received training. However, these authors indicated that no government body provided or facilitated any training. In the present study, the veterinary doctors, extension officers, and health assistants who shared information about meat handling requirements with vendors are employed under the different Kamuli administrative units (district, town councils, and sub-counties) of the government. The percentage of trained butchers (96.6%) in the present study is much higher than the 56.7% reported by Lamunu et al. (2022) in Wakiso district, the 31% by Kunyanga et al. (2021) in Meru, Kitui, and Tharaka Nithi counties in Kenya, the 62.5% by Zerabruk et al. (2019) in Ethiopia, and the 30.6 and 14.1% reported by Chepkemoi et al. (2015) in Nairobi and Isiolo counties in Kenya respectively.

To understand if the information disseminated to

vendors covers the US 736:2019 standard's requirements, the vendors were asked what they knew about the food safety requirements or requirements to run a butchery. Sixty-nine percent ($n = 41$) emphasized that personal hygiene was a requirement for operating a butchery. This was followed by sanitation at the butchery (32.2%, $n = 19$), medical forms (32.2%, $n = 19$), permits or documentation such as letters or licenses from authorities (23.7%, $n = 14$), tools and approved weighing scales (20.3%, $n = 12$), beef inspection (10.2%, $n = 6$), customer care (6.8%, $n = 4$), slaughtering by designated people (6.8%, $n = 4$), ensuring that beef is fresh (5.1%, $n = 3$) and that it is packaged properly (5.1%, $n = 3$), separating meat products sold at the butchery (1.7%, $n = 1$), displaying beef in a net (1.7%, $n = 1$), and not receiving beef returned by customers (1.7%, $n = 1$). These results contradict the findings by Jeffer et al. (2021) who reported that only 2% of meat handlers in Kampala were aware of the requirements for operating a butchery business. At least one-third of beef vendors in this study were aware of requirements for personal hygiene (Section 8), sanitation (Sections 6 and 7), and possession of a medical form (Section 4C). However, none of the vendors mentioned requirements for construction (Section 5), and less than 8.3% mentioned using a net or fly-proof screen (Section 7.1.1), separating meats of different kinds (Section 7.1.2), and selling fresh meat (Section 9.4). The limited knowledge of the vendors about food safety requirements can explain the low compliance levels noted. Although personal hygiene and sanitation of premises were mentioned by more than 32% of beef vendors, there is still a need for improvement especially with the use of personal protective coats, having more than one person at the butchery, keeping flies away from beef during transportation and display at butchereries.

CONCLUSION AND RECOMMENDATIONS

This study aimed to determine if beef vendors in Kamuli district in Uganda complied with the minimum food safety requirements of US 736:2019 standard and to identify supportive services available to beef vendors in complying with these requirements. Overall, beef vendors complied with the requirements to sell meat that has been inspected (Section 10.1), obtain meat from an approved slaughterhouse (Section 10.3), store meat by either refrigeration or hanging it inside the butchery for less than 36 hours (Section 9.4), clean butchereries every day (Section 9.5), and have short or covered hair, short fingernails and no jewelry (Sections 8.6, 8.7 and 8.8). Vendors' compliance could be attributed to the fact that the recommended practices were affordable and to the efforts of the Public Health and Veterinary departments of Kamuli district, who provided inspection services at the slaughterhouses and at the butchereries, and informed.

vendors about food safety requirements on different occasions

However, beef vendors violated the requirements to protect beef from flies during transportation and display at the butchery (Section 7.1.1), wear protective clothing (Section 8.5), construct butchery walls and floors using non-absorbent material (Section 5), not eat food from inside the butchery (Section 8.9), and have a separate person to handle money at the butchery (Section 8.3). Violations of the food safety standards could have resulted from limited knowledge of those specific parts of the standard, limited understanding of why safe food handling practices are important, and the extra financial expenses incurred to follow requirements. The violations reported in this study compromise the safety of beef, increase the risk to public health, and could have contributed to the high diarrhoea disease outcomes reported within the Busoga region (UBOS and ICF, 2018).

Violations could be mitigated through providing culturally appropriate food safety educational interventions covering food safety requirements in the US 736:2019 standard, and the importance of following the recommended practices. Educational interventions should be designed to use more visuals and demonstrations such that vendors with limited education and speaking Lusoga language can understand the safe meat handling practices. A waterproof coating could be added to the wooden walls and floors of butchereries to make them cleanable with water and soap for vendors who may not afford smooth cemented surfaces. Since vendors are already practicing sharing of resources, a district-owned refrigerated truck can be adopted for cold transportation and distribution of beef from the slaughterhouse to the butchereries, starting with the Municipal council which has the highest number of butchereries in Kamuli district.

Based on the fly control findings from this study, future research can investigate the effectiveness of selling only fresh meat, smoking wood, charcoal, or mosquito coils, maintaining cleanliness, and using the recommended fly screens to keep flies away from raw meat at butchereries, while making sure customers can see the displayed meat. Other techniques that serve the same purpose of inexpensively and safely controlling flies at butchereries also could be investigated.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Evaluation of thermosonication in the inactivation of lipoxygenase in hydrosoluble soy extract

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Hydrosoluble soy extract (HSE) is nutritious and highly perishable, requiring it to be submitted to an adequate conservation method. Ultrasound combined with heat (thermosonication) can be an alternative capable of reducing the undesirable effects caused by the conventional thermal treatment in this product. The objective of this work was to evaluate the effect of thermosonication on the inactivation of the enzyme lipoxygenase (LOX) in HSE under 2 ultrasonic amplitude conditions (70 and 90%). Fresh and treated HSE samples were characterized in terms of color parameters, pH, soluble solids and total phenolic content. Temperature was the most important factor in reducing LOX residual activity (RA). Highest LOX inactivation condition (RA = 2.14%) occurred in the range of 90% ultrasonic amplitude at 83°C for 3 min. In this condition, the specific acoustic energy (SAE) in thermosonicated sample was 596.7 mW/mL. Thermosonication has the potential to minimize the phenolic losses (Total Phenolic Content = 35.25 ± 0.25 mg/100 mL), when compared to heat treatment (Total Phenolic Content = 13.48 ± 0.06 mg/100 mL). Thermosonication has an interesting potential in maintaining the nutritional value of the HSE.

Key words: Hydrosoluble soy extract, enzyme inactivation, lipoxygenase, thermosonication, ultrasound.

INTRODUCTION

Hydrosoluble soy extract (HSE), an aqueous emulsion resulting from the hydration of soybeans, is a food recognized for its nutritional richness and low-fat content, as well as the absence of lactose and cholesterol. The consumption of this drink has increased significantly, especially among lactose-intolerant consumers,

vegetarians, vegans and/or those seeking healthier diets (Kubo et al., 2021; Kumar et al., 2021).

Due to the nature of its composition, neutral pH, high water activity and the presence of several metabolic enzymes such as lipoxygenase (LOX), HSE is naturally susceptible to enzymatic and microbiological degradation

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and is therefore a highly perishable product. Thus, to ensure food safety and extend its shelf life, this food must be subjected to adequate conservation processes immediately after it is obtained (Alhendi et al., 2017). Usually, the principle of heat treatment applied to HSE is based on inactivating LOX (biological indicator) due to the undesirable effects of this enzyme on the product, such as rancidity, and its high thermal resistance (Kubo et al., 2021; Kumar et al., 2021).

Lipoxygenase (linoleate: oxygen oxidoreductase, EC 1.13.11.12) catalyzes the hydroperoxidation reaction, that is, the addition of molecular oxygen to polyunsaturated fatty acid molecules containing *cis,cis*-1,4-pentadiene. These undesirable reactions on fatty acids promote their degradation and the release of volatile and nonvolatile compounds responsible for undesirable flavors (Alhendi et al., 2017).

Vegetable LOXs are monomeric proteins of approximately 95-100 kDa, with the metal site octahedrally coordinated by five amino acid side chains and a water or hydroxyl ligand. In the case of plant LOXs, these residues are always three histidines, one asparagine and one isoleucine (Ji et al., 2022). LOX isoenzymes are globular proteins that contain a nonheme iron atom, constituting a prosthetic group essential for enzymatic catalysis. When catalyzing the addition of oxygen to linolenic and linoleic acids, which are fatty acids present in HSE, formation of hydroperoxides involved in deteriorative reactions occurs, resulting in the formation of volatile products that modify the original flavor and generate characteristic rancid odor and taste (Ji et al., 2022; Lampi et al., 2020).

Heat treatment is a commonly used method for HSE conservation to ensure food safety and extend shelf life. However, heat can also cause undesirable changes, such as protein denaturation, amino acid deterioration and reactions that ultimately diminish the beverage's sensory quality and nutritional value (Amitabh et al., 2017; Hao et al., 2023).

Thus, given the undesirable effects and consumer eagerness for high-quality food, there is a need for alternative conservation techniques that minimize the damage caused by traditional heat treatment. In this context, several emerging technologies, both thermal and nonthermal, are already being investigated in soybean water-soluble extracts, including ohmic heating (Amitabh et al., 2017), microwave (Kubo et al., 2021; Kumar et al., 2021; Vagadia et al., 2018), high pressure (Andrés et al., 2016), and pulsed light (Alhendi et al., 2017) treatments. Among the nonthermal technologies, ultrasound is highlighted as a promising technique, which, despite presenting satisfactory results in several products, has not yet been investigated for soybean water-soluble extracts.

Ultrasound consists of sound waves that have a frequency above 16 kHz and are not detected by the human ear. It is an emerging technology that ensures

food quality and preservation through minimal processing, usually at room or mild temperatures. Enzyme inactivation through ultrasound is related to the phenomenon of cavitation, which involves the formation, growth, and implosion of bubbles when the liquid medium is subjected to sound waves. The collapse of cavitation bubbles leads to localized mechanical and chemical effects (temperatures up to 5,000 K and pressures up to 50,000 kPa), which lead to enzymatic inactivation (Khadhraoui et al., 2021).

When used alone, ultrasound may not be as efficient, so to achieve significant results, it is convenient to combine it with other conservation techniques, such as heat and pressure treatment. Thermosonication involves the simultaneous use of low-frequency ultrasound waves and milder temperatures than those used in conventional thermal processing.

When combined with heat, ultrasound can have a synergistic effect, accelerating the microbiological and enzymatic inactivation rates and, consequently, reducing the rigor of traditional heat treatments. Thus, in addition to decreasing the temperature and/or processing time, this technique has the potential to minimize undesirable changes in the nutritional and sensory quality of the treated food (Dolas et al., 2019). Several studies have revealed the potential of thermosonication in enzymatic inactivation in products such as coconut water (Ribeiro et al., 2017), grapefruit juice (Manzoor et al., 2021; Xu et al., 2023), and hazelnut milk (Atalar et al., 2019).

Therefore, the objective of this work was to evaluate the effect of thermosonication on inactivation of the enzyme lipoxygenase in hydrosoluble soy extract under different ultrasonic amplitudes.

MATERIALS AND METHODS

Preparation of HSE

Hydrosoluble soy extract was obtained as described in (Kwok and Niranjana, 1995) with some modifications. A hundred grams (100 g) of soybeans purchased from the local market in Lavras (Minas Gerais, Brazil) were weighed and soaked in distilled water for 20 h at a ratio of 1:3 (m/v). The remaining water was then discarded, and the grains were washed with distilled water. Then, the swollen beans were ground in an industrial blender for 2 min, with the addition of water at a ratio of 1:3 (m/v). To remove the insoluble material, the extract obtained was manually filtered through nylon fabric and then centrifuged (Thermo Scientific, USA) at 4°C for 20 min at a speed of 10,000 × g. Finally, the supernatant was collected and used as the soybean water-soluble extract in the experiments. This extract was placed in amber glass bottles and kept under refrigeration (~5°C) until thermosonication and conventional thermal treatment.

Based on previous work in literature (Kwok and Niranjana, 1995; Kwok et al., 2002; Vagadia et al., 2018), the ranges of the independent variable temperature (°C) and time (min) of heat treatment on LOX inactivation in HSE were defined. The preliminary experiments were carried out in a 3×3 factorial design, with 3 temperature levels (70, 80 and 90°C) and 3 time levels (3, 5 and 8 min). All experiments were performed in 3 repetitions to calculate the mean and standard deviation (SD). The experimental results

Table 1. Factors and levels of the CCRD 2² for the minimization of LOX residual activity.

Factor	Level				
	$-\sqrt{2}$	-1	0	+1	$+\sqrt{2}$
Temperature (°C) (X ₁)	62	65	72.5	80	83
Time (min) (X ₂)	1.59	2	3	4	4.41

Source: Authors.

obtained in the 3×3 factorial design were submitted to analysis of variance (ANOVA) at the significance level of 5%.

Statistical analysis

A central composite rotational design (CCRD) 22 + 5 replicates at the central point's + 2 axial points (-1.41 and + 1.41) was used to optimize the independent variables temperature (X₁) and time (X₂) of thermosonication (Table 1) in order to minimize the residual activity of lipoxygenase in HSE. The CCRD 22 experiments were carried out under ultrasonic amplitudes of 70 and 90%.

The results obtained from the CCRD 2² were submitted to multiple linear regression analysis, and a quadratic polynomial model (Equation 1) was fitted to the data.

$$\hat{y} = \beta_0 - \beta_i X_i - \beta_{ii} X_i^2 + \beta_{ij} X_i X_j + e \quad (1)$$

Where \hat{y} is the residual enzyme activity (%), β_0 is the intersection of the model, X_i and X_j are the levels of the independent variables, e is the error and β_i , β_{ii} , and β_{ij} is the linear, quadratic and interaction coefficients, respectively.

The suitability of the models was assessed using the multiple determination coefficient (R²), the significance of the mathematical model (p<0.05), the model lack of fit (p>0.05) and the significance of the regression coefficients (p<0.05). Nonsignificant coefficients (p>0.05) were grouped into error e . All statistical analyses were performed using SAS[®] University Edition statistical software. To facilitate visualization and identification of the experimental conditions that minimize the residual activity of LOX in HSE, contour graphs were generated from the values predicted by the obtained mathematical model using SigmaPlot version 14.5.

Conventional heat treatment

To compare the synergistic effect of ultrasound and heat on LOX inactivation with the effect of conventional heat treatment, the hydrosoluble soy extract was subjected to traditional heat treatment (heat effect only) following the temperature and time conditions evaluated in the CCRD 2².

Initially, the samples were preheated to the temperature defined by the factorial design for each treatment by immersing a glass beaker containing 50 mL of HSE in a water bath (Model Q215S2 - Quimis, Brazil) at 90°C according to the established experimental conditions (Table 1). The temperature was controlled using a thermometer immersed in the liquid. Once the working temperature was reached, the beaker containing the sample was transferred to a water bath set to the test temperature, where it was kept for the predetermined period of time (3, 5, and 8 min). After each treatment, the samples were immediately cooled in an ice bath to 4°C, and the enzymatic activity of LOX was determined.

The residual activity (RA, %) results obtained for the conventional heat treatment were submitted to regression analysis, and a

quadratic polynomial model (Equation 1) was fitted. All tests were performed in triplicate.

Thermosonication

Initially, the samples were preheated to the temperature defined by the experimental design (Table 1) for each treatment by immersing the glass beaker containing 50 mL of hydrosoluble soy extract in a water bath at 90°C. The temperature was monitored by means of a thermometer immersed in the liquid. After reaching the working temperature, the beaker containing the sample was transferred to a QSonica ultrasonicator (Ultronique, Brazil) operating at a frequency of 20 Hz and equipped with a titanium probe measuring 0.3 cm in diameter. Thermosonication was performed by immersing 0.5 cm of the probe in 50 mL of previously heated hydrosoluble soybean extract. The treatment was carried out for the period of time pre-established in the design (Table 1). The sample temperature was maintained throughout the thermosonication process through a jacketed beaker with water circulating at the working temperature. The sample temperature was monitored throughout the process with a thermometer. After each treatment, the samples were immediately cooled in an ice bath to 4 °C, and the enzymatic activity of LOX was determined.

Calculation of ultrasonic power and specific acoustic energy

To calculate the ultrasonic power applied to the samples, a glass beaker containing 50 mL of hydrosoluble soy extract at room temperature (25°C) was subjected to ultrasonic treatment under the two proposed amplitude conditions (70 and 90%) for 2, 3 and 4 min. With the aid of a thermometer and a stopwatch, the increase in temperature as a function of sonication time was recorded at intervals of 30 s. With these data, the power (W) and consequently the specific acoustic energy (mW/mL) for each tested time and amplitude condition were determined (Ribeiro et al., 2017). The potency of each treatment was determined according to Equation (2):

$$P(W) = m \times c_p \times \left(\frac{dT}{dt}\right) \quad (2)$$

Where P is power (W); m is the mass (g) of 50 mL of the hydrosoluble soy extract; c_p is the specific heat of the hydrosoluble soy extract (4649.6 J/kg °C), and $\frac{dT}{dt}$ is the rate of change in temperature during sonication (°C/s). The specific acoustic energy (SAE, mW/mL) under each condition was obtained through the ratio between the power and the sample volume (V, mL) according to Equation (3):

$$SAE = \frac{P}{V} \quad (3)$$

Lipoxygenase enzymatic activity

The lipoxygenase enzyme activity in the control and treated HSE was determined according to the methodology described in literature (Li et al., 2008). The control corresponded to HSE samples without any previous heat treatment (thermosonication or conventional heat treatment). Initially, 0.1 mL of each sample was pipetted into 25 mL test tubes and diluted with 19 mL of distilled water. The diluted solution was stored for later use.

The substrate solution was prepared immediately before carrying out the enzymatic activity analyses. This solution was composed of linoleic acid:ethyl alcohol:0.2 mol/L borate buffer (pH 9.0) (1:1:1,000 v/v), totaling 5 mL, which was mixed in 20 mL of 0.2 mol/L borate

Table 2. Preliminary experimental results of residual activity (RA) of LOX in HSE after thermal treatments.

Temperature (°C)	Time (min)	RA (%)
70	3	87.75 ± 1.62
70	5	75.64 ± 1.11
70	8	22.42 ± 1.68
80	3	3.14 ± 0.44
80	5	3.09 ± 0.50
80	8	2.13 ± 0.64
90	3	2.85 ± 0.55
90	5	2.14 ± 0.33
90	8	0.95 ± 0.28

The RA (%) result is mean ± standard deviation values.
Source: Authors.

buffer (pH 9.0) and 5 mL of distilled water. Then, 2 mL of this substrate solution and 0.95 mL of 0.2 mol/L borate buffer (pH 9.0) were pipetted into a quartz cuvette and mixed by inversion at 25°C.

Afterward, 0.05 mL of the diluted sample was added to the quartz cuvette and immediately mixed by inversion. The decrease in absorbance was observed for 3 min ($\Delta A_{234}/\text{min}$) in a VIS 190-1100 nm spectrophotometer (Drawell, DU-8200, China) at a wavelength of 234 nm. A unit of activity (U) was defined as the amount of enzyme required to produce a 0.001 decrease in optical density per minute (Li et al., 2008). The tests were carried out in triplicate. The enzyme activity was calculated according to Equation (4):

$$EA = \frac{(\Delta A_{234}/\text{min})_{\text{enzyme}}}{0.001 \times 0.1} \times f \quad (4)$$

Where EA is the enzymatic activity (U/mL) of lipoxygenase in the HSE; f is the HSE sample dilution factor; and $(\Delta A_{234}/\text{min})_{\text{enzyme}}$ is the maximum speed of enzyme activity (U). LOX inactivation was evaluated by determining the residual activity (RA, %) of lipoxygenase in the HSE, defined according to Equation (5):

$$RA = \frac{EA}{E_0A} \times 100 \quad (5)$$

Where EA is the enzymatic activity (U/mL) of lipoxygenase in the HSE sample after treatment and E_0A is the initial enzymatic activity (U/mL) of lipoxygenase in the HSE sample before treatment, both calculated according to Equation (4):

Physicochemical analysis

The physicochemical characteristics and the total phenolic content of the optimal experimental conditions obtained from the CCRD were determined.

The pH of the HSE samples was determined with a benchtop pH meter (MS Tecnoyon, Brazil). The soluble solids content (°Brix) was determined by measuring the refractive index using a digital refractometer (Atago, Brazil). The colorimetric parameters L^* (luminosity), a^* (red-green axis coordinate) and b^* (blue-yellow axis coordinate) were measured in a colorimeter (Konica Minolta, Japan). The total color difference (E^*) was calculated according to Equation (6) (Oladunjoye et al., 2021).

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (6)$$

Where $^{\circ}$ indicates the variation in the parameters, such that $\Delta L^* = (L^* - L_0^*)$ is the difference between the luminosity of the thermosonicated samples and the control; $\Delta a^* = a^* - a_0^*$ represents the red (positive value) and green (negative value) color intensities of the thermosonicated samples and the control; and $\Delta b^* = b^* - b_0^*$ represents the intensities of the yellow (positive value) and blue (negative value) colors of the thermosonicated samples and the control.

Total phenolic content

The total phenolic content (TPC) was determined using the colorimetric method described by Rodríguez-Roque et al. (2013). Briefly, an aliquot of 0.5 mL of HSE (without heat treatment, subjected to thermosonication or conventional heat treatment) was mixed with 0.5 mL of Folin-Ciocalteu reagent and 10 mL of Na_2CO_3 (20% m/v). The volume of the mixture was brought to 25 mL with distilled water using a volumetric flask. The resulting solution was kept in the dark at room temperature (25°C) for 1 h. Then, the absorbance of the samples was measured at 725 nm. The calibration curve for determining the total phenolic content was constructed using gallic acid (GA) as a standard at concentrations from 20 to 75 mg/100 mL. The results were expressed in mg of gallic acid equivalent (GAE) per 100 mL of the extract.

RESULTS AND DISCUSSION

The average results obtained in the preliminary tests using the 3x3 factorial design is presented in Table 2. These results were submitted to ANOVA, followed by regression analysis; the mathematical model obtained for the inactivation of LOX in HSE as a function of thermosonication time and temperature is presented in Equation 7. The adjusted mathematical model was significant ($p < 0.05$) and presented significant coefficients ($p < 0.05$), a nonsignificant lack of fit ($p > 0.05$) and R^2 value higher than 0.94.

$$\hat{y} = 2450.4852 - 54.0057 x_1 - 59.0509 x_2 + 0.6821 x_1 x_2 + 0.2963 x_1^2 \quad (7)$$

Treatments that used temperatures at or above 80°C were found to almost completely inactivate LOX in the HSE, and at 80°C for 8 min, the enzyme showed residual activity similar to that found at 90°C for 5 min. Moreover, at 70°C, with the shortest time period (3 min), LOX presented high resistance to inactivation, and its residual activity remained very high. Although lipoxygenases can be inactivated above 60°C, the effective reduction in their activity is related to processing conditions and their isoforms (Kubo et al., 2021).

For the subsequent step, a temperature range of 62 to 83°C and a time of 1.59 to 4.41 min were selected to optimize the operating conditions of thermosonication, aiming to minimize the LOX residual activity. Importantly, the temperature selection aimed to range from low to high LOX reduction to assess the synergistic potential of ultrasound in inactivating this enzyme.

Table 3. Residual activity of LOX (%) in HSE obtained for treatments submitted to conventional thermal treatment and thermosonicated in the ranges of 70 and 90% amplitude.

Temperature (°C)	Time (min)	Thermal treatment	70%	90%
65	2	72.50	74.19	63.64
65	4	68.71	64.51	60.00
80	2	7.00	2.35	3.87
80	4	3.48	3.13	2.90
62	3	89.93	76.67	70.0
83	3	3.09	3.75	2.14
72.5	1.59	44.12	43.14	24.24
72.5	4.41	11.76	11.76	6.36
72.5	3	33.82	17.31	16.13
72.5	3	35.29	19.23	16.67
72.5	3	23.53	21.15	20.00
72.5	3	28.57	15.38	15.15
72.5	3	35.71	23.53	15.15

Source: Authors.

Optimization of thermosonication operating conditions

In this optimization step, the criterion used to choose the optimal ultrasonic conditions was the greatest inactivation of LOX in the HSE; that is, the selected treatments were those that minimized the residual LOX activity. Additionally, the experimental conditions evaluated with CCRD 2² for LOX inactivation in HSE were reproduced to apply a conventional heat treatment (heat effect only) to the HSE. Thus, it was possible to evaluate the synergistic effect of ultrasound and heat in the inactivation of LOX compared to the effect of a traditional heat treatment.

The experimental results of RA obtained from the CCRD 2² for ultrasonic amplitudes of 70% and 90% and conventional thermal treatment are presented in Table 3.

The residual activity of lipoyxygenase obtained from the CCRD 2² for 70 and 90% ultrasonic amplitudes, as well as in traditional heat treatment, was submitted to regression analysis (Table 4). The mathematical models presented in Table 5 describe the relation between the independent variables (time and temperature) considering only the significant terms ($p < 0.05$).

According to Table 5, for conventional thermal treatment and thermosonication at amplitudes of 70% and 90%, the parameters of time and temperature significantly affected ($p < 0.05$) the residual activity of lipoyxygenase; the temperature (X_1) had a quadratic and linear effect, while time (X_2) had only a linear effect. From the models obtained to predict the residual activity of LOX, contour graphs were constructed (Figures 1 and 2) for all the thermal treatments evaluated.

By analyzing the thermosonication and conventional thermal treatment curves (Figures 1 and 2), it can be

seen that temperature appears to be the most important factor in reducing enzymatic activity. At higher temperatures, a synergistic effect was observed between heat and sonication for enzyme inactivation because at a temperature of approximately 77°C, for example, the reduction achieved by conventional treatment was 80%, while with thermosonication at an amplitude of 90, 90% inactivation was found.

From analysis of Figures 1 and 2, it was possible to observe that there was no increase in the reduction of enzymatic activity at 70% sonication amplitude compared to conventional thermal treatment. At 90% ultrasonic amplitude, a synergistic effect was observed. This finding demonstrates that although no decrease in enzymatic activity occurred at 70% amplitude compared to that with conventional thermal treatment, at higher amplitudes, such as 90%, the combination of ultrasound and heat had a synergistic effect on LOX enzyme inactivation in HSE.

The difficulty encountered when inactivating LOX at temperatures below 72.5°C may be related to the existence of at least 3 to 4 isoenzymes that differ in their thermal stability. Thus, while the thermolabile fraction can be rapidly inactivated, complete inactivation of the heat-resistant fraction is more difficult, causing the residual activity of the enzyme in the HSE to be high even after applying heat treatment (Kubo et al., 2021).

High temperatures can cause changes in the structure of the enzyme, such as breaking hydrogen bonds and denaturation. On the other hand, ultrasound forms cavitation bubbles capable of altering the structure of proteins by breaking bonds of the peptide chain, generating free radicals. The enzymatic inactivation caused by thermosonication is attributed to the effect between heat and mechanical damage that leads to

Table 4. Regression Analysis from CCRD 2².

SV	SS			DF	MS			F calculated			p value		
	TT	70%	90%		TT	70%	90%	TT	70%	90%	TT	70%	90%
X ₁	8037.66	6991.14	5668.00	1	8037.66	6991.14	5668.00	124.65	142.34	284.56	<0.00	<0.00	<0.00
X ₂	262.28	354.82	84.30	1	262.28	354.82	84.30	4.07	7.22	4.23	0.09	0.04	0.09
X ₁ × X ₂	0.02	27.35	1.78	1	0.02	27.35	1.78	0.00	0.56	0.09	0.99	0.48	0.78
X ₁ × X ₁	445.59	770.06	913.14	1	445.59	770.06	913.14	6.91	15.68	45.84	0.04	0.01	0.00
X ₂ × X ₂	12.61	145.12	38.15	1	12.61	145.12	38.15	0.20	2.95	1.92	0.67	0.14	0.22
Lack of Fit	8.29	15.63	84.88	1	8.29	15.63	84.88	0.13	0.32	4.26	0.73	0.59	0.09
Error	386.89	294.70	119.51	6	386.89	49.11	19.92						
Total	9153.35	8598.82	6909.77	12									

SV, Source of variation; X₁, temperature (°C); X₂, time (min); TT, Thermal treatment; SS, Sum of squares; DF, Degree of freedom; MS, Mean square. Source: Authors.

Table 5. Prediction models for LOX residual activity as a function of temperature (X₁, °C) and time (X₂, min) for the conventional thermal treatment and thermosonication at different amplitudes.

Attribute	Mathematical models
Conventional thermal treatment	$\hat{y} = 1111.2807 - 25.0259 X_1 - 6.6117 X_2 + 0.1433 X_1^2$
Thermosonication - 70%	$\hat{y} = 1319.8373 - 31.2767 X_1 - 6.6597 X_2 + 0.1884 X_1^2$
Thermosonication - 90%	$\hat{y} = 1365.3930 - 33.3117 X_1 - 3.7483 X_2 + 0.2051 X_1^2$

Source: Authors.

protein denaturation by depolymerization and alteration of its tertiary structure conformation (Wang et al., 2023).

The specific mechanism of enzyme inactivation during sonication may be due to a single or combination of several chemical and physical effects occurring simultaneously. Ultrasonic inactivation mechanisms are specific to the enzyme under investigation and depend on its amino acid composition and conformational structure. For example, lipoxygenase appears to be inactivated by a mechanism mediated by free

radicals and by protein denaturation (Ji et al., 2022; Khadhraoui et al., 2021). These radicals, generated during sonication, play an important role in enzymatic inactivation, as they disturb hydrophobic interactions and intramolecular hydrogen bonds, which play important roles in protein stability (Ampofo and Ngadi, 2022; Tian et al., 2004).

Amino acids, such as tryptophan, tyrosine, histidine and cysteine, present in soybeans, are particularly susceptible to degradation by hydroxyl and superoxide free radicals. In addition to proline,

the amino acids leucine, isoleucine, lysine, cysteine and glutamic acid easily form peroxides when reacting with OH· radicals (Wang et al., 2023). The free radicals formed react with the enzyme's amino acid residues, making them unable to participate in stabilization of the molecule, bind to the substrate and exert its catalytic function (Ampofo and Ngadi, 2022; Tiwari and Mason, 2011). At the same time, the presence of OH· radicals diminishes the antioxidant properties of foods and can cause off-taste in some foods. Free hydroxyl radicals, OH·,

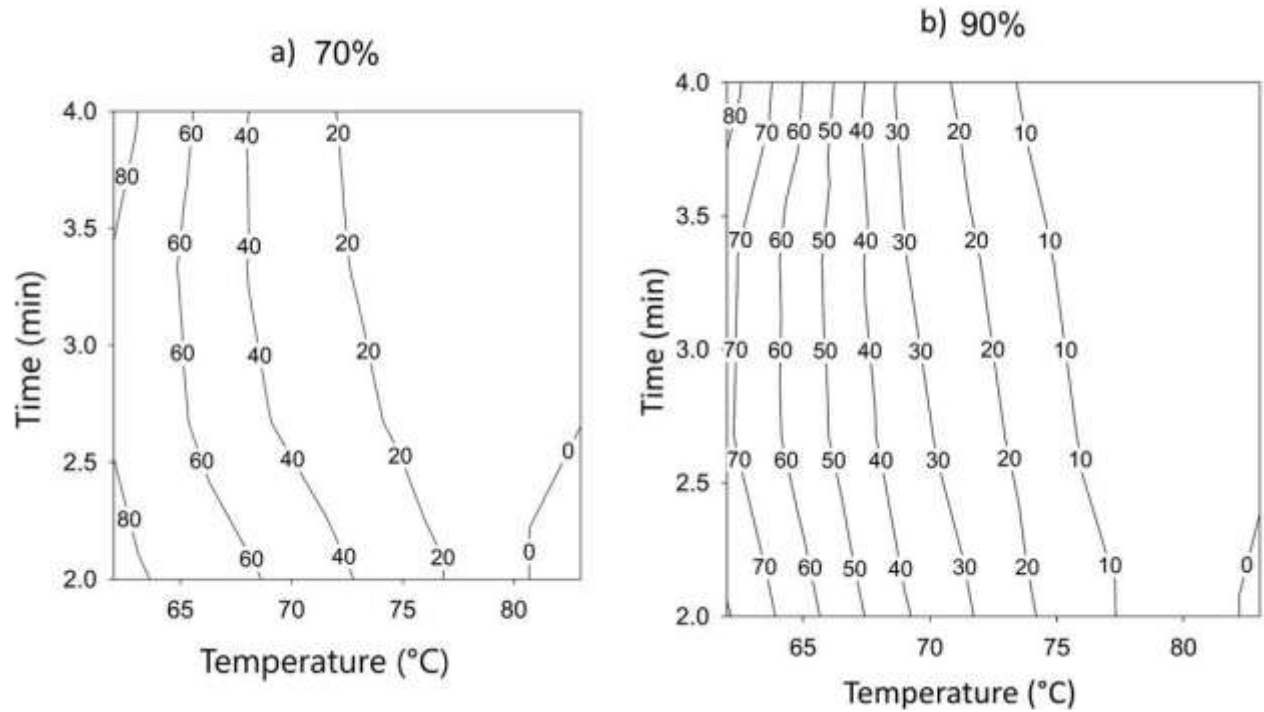


Figure 1. Contour plot of residual activity (%) of LOX as a function of thermosonication time (min) and temperature (°C) in the amplitudes of (a) 70% and (b) 90%.
Source: Authors.

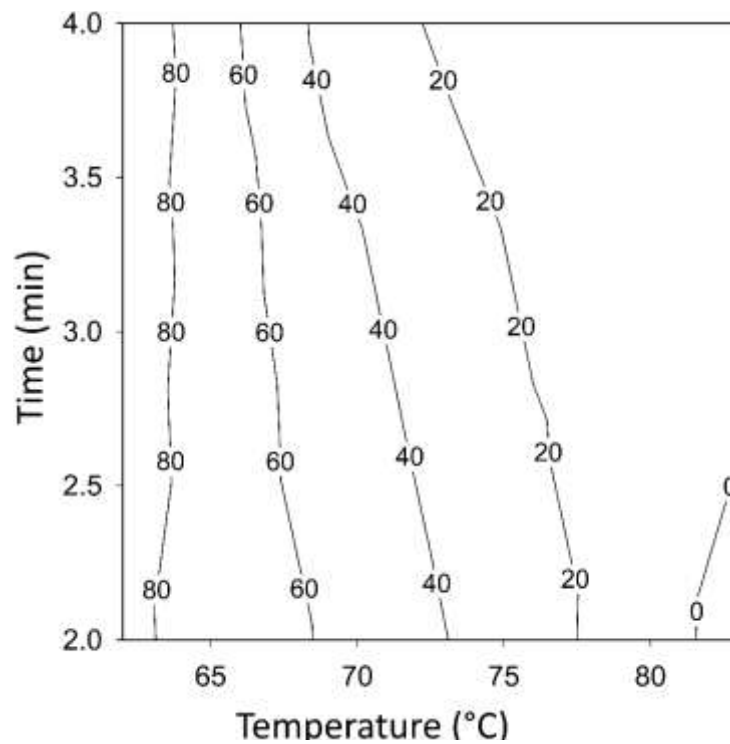


Figure 2. Contour plot of residual activity (%) of LOX, as a function of time (min) and temperature (°C) applied in conventional thermal treatment.
Source: Authors.

Table 6. Specific acoustic energy (SAE) of thermosonicated samples.

Treatment	Amplitude (%)	Time (min)	SAE (mW/mL)
1	70	2	612.8 ^a
2	70	3	583.8 ^{ab}
3	70	4	582.7 ^{ab}
4	90	2	610.0 ^a
5	90	3	596.7 ^a
6	90	4	561.0 ^b

Means followed by the same letter do not differ statistically from each other at the level of 5% probability by t-student test.

Source: Authors.

Table 7. Chemical parameters of fresh HSE (control) and HSE submitted to conventional thermal treatment and thermosonication at 70 and 90% of amplitude, both at 83°C for 3 min.

Treatment	L*	a*	b*	ΔE*	pH	°Brix	TPC (mg/100 mL)
Fresh HSE	57.99 ± 2.29 ^a	1.58 ± 0.52 ^a	10.67 ± 2.19 ^a	-	6.49 ± 0.72 ^b	6.50 ± 0.36 ^b	49.61 ± 0.17 ^a
TT	58.40 ± 0.87 ^a	1.26 ± 0.60 ^{ab}	11.26 ± 0.43 ^a	4.15 ± 0.86 ^a	5.99 ± 0.15 ^b	7.01 ± 0.10 ^a	13.48 ± 0.06 ^d
70%	56.79 ± 1.47 ^a	0.76 ± 0.30 ^b	10.00 ± 0.62 ^a	4.18 ± 0.71 ^a	6.75 ± 0.50 ^a	7.30 ± 0.24 ^a	35.25 ± 0.25 ^b
90%	57.80 ± 2.27 ^a	1.13 ± 0.66 ^{ab}	10.58 ± 0.37 ^a	3.83 ± 0.59 ^a	6.10 ± 0.11 ^b	7.03 ± 0.20 ^a	28.35 ± 0.12 ^c

Means followed by the same letter do not differ statistically from each other at the level of 5% probability by t-Student test. TT: Thermal treatment. TPC: Total phenolic content. L*: luminosity. a*: red-green axis coordinate. b*: blue-yellow axis coordinate. ΔE*: total color difference.

Source: Authors.

are highly reactive species that have a very high redox potential and represent strong oxidants that can react quickly with most amino acids (Rodríguez-Rico et al., 2022). Different studies have evaluated the LOX activity reduction or inactivation by thermosonication treatments (Manzoor et al., 2021; Xu et al., 2023).

The enzyme activity strongly depends on the ultrasonic intensity, which can be observed at temperatures below 70 °C; the reason is that the samples thermosonicated at the highest amplitude (90%) showed higher enzymatic inactivation than those subjected to the same experimental conditions at the lowest amplitude (70%) (Islam et al., 2014). The increased effect of ultrasound by increasing the amplitude has been related to the increase in the effective size of the cavitation liquid zone and to the range of sizes of bubbles that cavitate at higher amplitudes (Vallath and Shanmugam, 2022).

The specific acoustic energy found for thermosonicated samples at different amplitudes (70 and 90%) and times (2, 3 and 4 min) was in the range of 561-613 mW/mL, with the highest power being obtained at 70% amplitude and a time of 2 min (Table 6).

The lowest residual activity of LOX in HSE (2.1%, Table 3) occurred under in one of the conditions with the highest acoustic power (90%, 3 min at 83°C). This result is attributed to the energy supplied to the liquid medium, which was able to break the hydrogen bonds and disrupt

the tertiary structure conformation of the enzyme, that is, expose its hydrophobic groups to the medium, leading to enzyme inactivation and aggregation (Islam et al., 2014).

Effect of thermosonication on the physicochemical characteristics and phenolic content

The physicochemical parameters L*, a*, b*, pH, total soluble solids and the total phenolic content of the samples treated by thermosonication at ultrasonic amplitudes 70 and 90% and by a conventional thermal treatment, both at a temperature of 83°C for 3 min, were evaluated to compare the rigors of heat treatment and thermosonication. These conditions were chosen because they resulted in the highest enzyme inactivation among all experimental treatments.

Fresh HSE corresponded to the hydrosoluble soy extract samples that were not subjected to any thermal treatment. The average results obtained for the physicochemical characteristics and the total phenolic content of the different samples were also compared by Student's t test, and the results obtained are shown in Table 7.

As presented in Table 7, among the parameters evaluated, only the L* and b* color parameters showed no significant difference (p>0.05) between treated

(thermal and thermosonication) and fresh HSE (control). For parameter a^* , there was a significant difference ($p < 0.05$) between samples thermosonicated at 70% amplitude and the control sample (without any treatment). For pH, a significant difference ($p < 0.05$) was observed between the control sample and those subjected to conventional thermal treatment and treated at 90% amplitude, while for the soluble solids content ($^{\circ}$ Brix) and total phenolic content, all treated samples differed significantly ($p < 0.05$) from fresh HSE.

Among the color parameters, only for the parameter a^* , related to the red-green axis, was a significant difference ($p < 0.05$) observed between the samples, and the treatments with ultrasound showed lower mean results than with the traditional heat treatment (Table 7). In this case, the thermosonicated samples showed a coloration closer to green (negative value) than to red (positive value); this result can be explained by the phenomenon of cavitation, which can induce changes in color by accelerating chemical reactions and increasing the rate of diffusion, dispersion, aggregate formation and particle breakage (Alcántara-Zavala et al., 2021; Xu et al., 2023). The average results obtained for the other color parameters were statically similar ($p > 0.05$), which suggests that the samples maintained the same luminosity and yellowish color. Based on the color parameters of the control sample, it can be concluded that the color difference between the samples (ΔE) was not significant ($p < 0.05$).

The samples differed significantly ($p < 0.05$) in relation to pH, mainly for samples thermosonicated at 70% amplitude, where a slight increase in this parameter was observed. Salve and colleagues (Salve et al., 2019) reported in their study with peanut milk that after thermosonication treatment, an increase in pH was associated with higher physical stability of the beverage.

The soluble solids content of the control sample showed a significant difference ($p < 0.05$) when compared to that of the other treatments; that is, the high temperature seems to be sufficient to break the cell walls or hydrolyze the polysaccharides (Maghsoudlou et al., 2016). Salve et al. (2019) observed that an increase in $^{\circ}$ Brix was related to an increase in protein solubility. According to Table 7, compared with samples subjected to the conventional thermal treatment, the thermosonicated samples presented a significantly lower reduction in the total phenolic content. Based on these data, although high temperatures reduced the total phenolic content of the water-soluble soybean extract with both conventional heat treatment and thermosonication, ultrasound still had higher potential to preserve these bioactive compounds. Jabbar et al. (2015) observed a behavior similar to that in the present article because when the ultrasound processing temperature increased, the loss of phenolic compounds in carrot juice also increased, but this loss was lower than that with traditional heat treatment.

Phenolic compounds are incorporated into vacuoles in soluble form or bound to the cell wall (Atalar et al., 2019). The basic principle behind the increased phenolic content involves cavitation in the food components and the pressure exerted during this process, which disrupts cell walls, making it easier to release phenolic compounds bound to the soybean matrix. It has been reported that an increase in the TPC by thermosonication occurs because the concentration of individual phenolic compounds, such as flavonoids, can also increase because of increased hydroxylation of molecules due to the formation of OH-radicals during ultrasound treatment (Ampofo and Ngadi, 2022; Cui et al., 2014).

Atalar et al. (2019) observed that thermal treatment at 85°C for 2 min led to a significant reduction in the TPC in hazelnut milk from 162 μg GAE/g to 150.74 μg GAE/g, while the highest TPC value (178.82 μg GAE/g) was found for hazelnut milk thermosonicated at 75°C at 60% amplitude for 25 min, indicating that temperature had a significant effect on TPC levels.

Conclusion

In this work, the effect of thermosonication on the inactivation of LOX in hydrosoluble soy extract was evaluated, and the synergistic effect of ultrasound and heat was compared with that of conventional thermal treatment in reducing the residual enzyme activity. A central composite rotational design (CCRD) was employed to study the influence of the independent parameters temperature and thermosonication time on the residual activity (RA) of LOX in HSE. Subsequently, the synergistic effect of ultrasound and heat on LOX inactivation was evaluated and compared to that of conventional heat treatments. Finally, HSE samples were characterized by physicochemical analysis and the total phenolic content.

At the highest amplitude (90%), a greater reduction in lipoxygenase enzyme activity in the hydrosoluble soy extract was observed, but compared to that of the traditional heat treatment; the synergistic effect of ultrasound with heating was not as significant. The condition with the highest LOX inactivation (RA=2.34%) was at 70% amplitude (80°C/2 min).

Regarding color, the thermosonicated samples did not show any significant difference relative to the heat-treated sample, but the pH showed a significant increase at 70% amplitude when compared to that of the other treatments. The soluble solids content did not differ significantly between the treatments, whereas a higher total phenolic content was observed in thermosonicated samples, especially at 70% amplitude. Further studies exploring other conditions, especially at higher acoustic energy levels, are strongly recommended. An interesting potential of thermosonication to maintain the nutritional value of the HSE, particularly the content of total

phenolics, was verified in this study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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