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Full Length Research Paper

Handling practices and microbiological quality of *kayabo* - salted sun-dried Nileperch *Lates niloticus* from Lake Victoria, Tanzania

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This study assessed handling practices and microbiological contamination of salted sun-dried Nile Perch, *Lates niloticus*, commonly known as *Kayabo* in Tanzania. The processors of *Kayabo* were small-scale processors located at Kanyama and Mwaloni, Mwanza. Mixed methods (observations, face-to-face interviews, and microbiological sampling) were used to assess handling practices of processors and microbiological quality of *Kayabo*. Hierarchical cluster analysis on handling practices of *Kayabo* processors produced three distinct clusters of 15 (Cluster I), 10 (Cluster II), and 5 (Cluster III) processors. In general, the majority of processors had inadequate training on appropriate food handling, lack of hygienically designed facilities (building and cooling), poor sanitation, and the use of low-quality raw materials (rejects from industrial processing units). Assessment of microbiological quality of *Kayabo* indicated a significant ($P < 0.05$) variation in Total Viable Counts, TVC (2.08 - 8.68 Log CFU/25 g), and *Staphylococcus aureus* ($< 1-5.49$ log CFU/25 g) among the clusters. About 80% (12/15) and 42% (5/12) of the processors in 'Cluster I' exceeded the legal limits for TVC (5 Log CFU/25 g) and *S. aureus* (3 Log CFU/25 g), respectively. Strikingly, *Escherichia coli* and *Listeria monocytogenes* were below the quantification limits. Intervention measures for improvement on the raw materials, structure, and infrastructure, training, and adoption of good practices across the value chain are urgently needed to ensure the quality and safety of *Kayabo*.

Key words: Fish, salted sundried Nile perch, handling practices, microbiological quality, *Kayabo*, Lake Victoria.

INTRODUCTION

The Tanzanian fishery sector is among the leading (154.5 Million USD) exporting sectors in the country. It is the source of income and contributes to 1.3% of Gross Domestic Product, GDP (LVFO, 2009; URT, 2013; FAO, 2014). The sector employs more than 400,000 full-time

artisanal fishermen and more than 4 million people in various fisheries-related activities (URT, 2013). About 30% of the total animal protein requirements in the country come from fish (URT, 2013). Lake Victoria contributes to 85% of the total export of fish and fish

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products (URT, 2013). Although the Lake has more than 290 fish species, only Nile perch (*Lates niloticus*), Tilapia (*Oreochromis niloticus*), and Sardines (*Rastrineobola argentea*) are of commercial importance (Abila, 2000; Akande and Diei-Ouadi, 2010; Kabahenda et al., 2009). The Nile perch is the only species from Lake Victoria industrially processed for the export market, whereas other species are processed solely for the domestic market (Kussaga et al., 2014; Medard et al., 2019).

Although fish processing companies for the export market enjoy strict control and monitoring of the food safety hazards, fish processing units for the domestic market like *Kayabo* processors are barely controlled (Kussaga et al., 2014; Baniga et al., 2019). Processors for the domestic market are small-scale with limited knowledge of proper fish handling and inadequately designed facilities to ensure the quality and safety of the products (Baniga et al., 2019). Processors use traditional processing techniques, particularly curing and sun-drying. When fish is salted and dried, it can have a shelf life of more than a year. *Kayabo* processing is mainly done by women and involves removal of guts, swim-bladder, and scales, before salting (Medard et al., 2019). Even though such traditional processing techniques are cheap and easy to operate, they expose the products to various food safety hazards.

Fish contains 70-84% water, 15-24% protein, 0.1-2.2% fat, and 1-2% minerals (Ghaly et al., 2010). Fish is an excellent dietary source of highly unsaturated fatty acid (UFA) and polyunsaturated fatty acid (PUFA), especially the omega-3 fatty acids, Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) (El-Sherif and Abd El-Ghafour, 2016). Unless properly preserved or processed, fish is a highly perishable product that could be rendered unfit for human consumption within 12 h of capture under tropical conditions (Ghaly et al., 2010). Immediately after a fish dies, several physiological and microbial processes occur (Davies, 2009; Jinadasa, 2014). The quality and safety of fish is affected by capture method, processing, and handling conditions along the chain (Quang, 2005; Amos, 2007; Akande and Diei-Ouadi, 2010). However, the fish processing value chain for the domestic market is not well regulated.

Although the raw materials for the domestic-market oriented fish processors are tilapia and sardines, the supply is not yet sufficient and of low quality (as there is no cooling and hygienic handling practiced by most of the artisanal fisheries). Consequently, the small-scale *Kayabo* processors purchase rejects (undersize or low quality fish and fish frames) from the export-market oriented fish processing companies (Kabahenda et al., 2009), which focus on Nile Perch processing. The use of low quality raw materials associated with inadequate handling and processing conditions could result in more food safety problems.

Various studies have been conducted to assess the quality and safety of fish and fish products for the export

market (Kussaga et al., 2014, 2017; Baniga et al., 2019); however, there is very restricted information on the quality and safety of fish or fish products like *Kayabo* for the domestic market. The status of premises and buildings of *Kayabo* processors has never been evaluated, limiting the opportunities for improvement. Moreover, it is not yet known to what extent consumers of processed fish by the domestic market-oriented units are exposed to various food safety hazards. Therefore, the purpose of this study was to investigate the handling practices and microbiological quality of *Kayabo* along the product value chain. The information generated from this study will be beneficial to consumers, small-scale processors, researchers, food control/competent authorities, and policymakers to set strategies for improving fish processing and handling practices to reduce food safety risks along the fish value chain.

MATERIALS AND METHODS

Characteristics of *Kayabo* processors involved in the present study

The present study was conducted in two municipalities of Mwanza City, namely: Nyamagana and Ilemela, where Kanyama and Mwaloni processing sites are located, respectively. Thirty *Kayabo* processors, 18 being micro-scale (<10 employees) and 12 small-scale (10-49 employees) participated in this study. These processors produced whole salted sun-dried Nile perch (commonly known as *Kayabo*), salted sun-dried Nile perch heads (also known as *Mapanki*), salted sun-dried Nile perch trims (chips), and sun-dried Nile perch bones for fish meal production. Face-to-face interviews, observation, and sampling for microbiological assessments were carried out. However, *Kayabo* was the only product sampled and analysed for microbiological hazards as it was the main product processed.

Assessment of hygienic practices

A structured questionnaire and observation checklist were used to assess processors' knowledge of manufacturing and hygienic practices (transportation, processing conditions, packaging, and storage of raw materials and products). The questionnaire contained both open and closed-ended questions. The questions were on location and building/equipment layout, source of water, raw materials, sanitation, and personal hygiene, control by food control authorities, packaging, and demographic information of the respondents. The study assessed thirty *Kayabo* processing companies; the interviewees were either operators or owners.

Assessment of microbiological contamination

Selection of microbiological parameters

Five microbiological parameters including indicators of general process hygiene (Total Viable Counts, TVC), fecal hygiene (total coliforms and *Escherichia coli*), personnel hygiene (*Staphylococcus aureus*), and food safety (*Listeria monocytogenes*) were selected and analyzed (Jacxsens et al., 2009; Kussaga et al., 2017). Testing for TVC aimed at assessing the quality of raw materials, effectiveness of the handling procedures and hygiene conditions

during processing, sanitary conditions of equipment and utensils, and time/temperature profile during storage and distribution. The natural habitat for *E. coli* is in the intestines of human and vertebrate animals (Berthe et al., 2013). Contamination of food with *E. coli* implies a risk that one or more enteric pathogens may have gained access to the food. Total coliforms indicate a higher probability that organisms of fecal origin are present and hence fecal contamination. The natural reservoirs for *S. aureus* are human skin, hair, and superficial mucous membranes (nose), the presence of large numbers indicates a possibility of enterotoxin production or fault in sanitary or production practices. The natural habitat of *L. monocytogenes* is in environments like soil, water, and fecal matter. Its presence in food is an indication of poor sanitation and animal control (FAO / CDR, 2013).

Sample collection and analysis

Systematic random sampling was used to take samples (whole *Kayabo*) from 30 processors. Each sample was inserted in a labelled sterile bag and then placed into a sterile cool box maintained at $<5^{\circ}\text{C}$ and transported in that condition to the accredited National Fish Quality Control Laboratory (NFQCL) for analysis. The samples that were not analysed on the same day of sampling were frozen at -18°C until analysed. Enumerations of TVC, total coliforms, *E. coli*, *S. aureus* were performed according to ISO 4833:2003 (ISO, 2003a), ISO 4831:2006 (ISO, 2006), ISO 7251: 2005 (ISO, 2005), ISO 6888 – 3: 2003 (ISO, 2003b), respectively. Detection of *L. monocytogenes* was as per ISO 11290-1:1996/Amd 1: 2004 (ISO, 2004) methods. The preparation of analytical reagents (dilutents) and media was according to the manufacturers' instruction(s) and the specific test method requirements.

The preparation of analytical samples was in accordance with US-FDA Bacteriological Analytical Manual (2001). Weighing of 25 g *Kayabo* samples was by an analytical balance, Adventurer TM PRO OHAUS made in China. Then 225 ml buffered peptone water (BPW) was added to each sample and blended for 1 min to homogenize the mixture by using a stomacher (Seward STOMACHER R 3500 Lab System). Serial dilutions were aseptically prepared; 1 ml was taken from each dilution and plated on Plate count Agar (PCA, for TVC), Violet Red Bile Agar (VRBA for *E. coli*), and Baird Parker Agar (BPA, for *S. aureus*). The MPN was used for analysis of total coliforms. The incubation conditions were $30 \pm 1^{\circ}\text{C}$ for 72 ± 2 h for TVCs, $44 \pm 1^{\circ}\text{C}$ for 24 ± 2 h for *E. coli*, $37 \pm 1^{\circ}\text{C}$ for 48 ± 2 h for *S. aureus* and $37 \pm 1^{\circ}\text{C}$ for 48 ± 2 h for total coliforms. For the detection of *L. monocytogenes*; 225 ml of Half Fraser broth for the primary enrichment were added to 25 g of each *Kayabo* sample and the mixture blended by using the stomacher for 1 min. Then, the homogenate was incubated at $30 \pm 1^{\circ}\text{C}$ for 24 ± 2 h.

Interpretation of microbiological results

Tanzanian standards (TZS), East African Standards (EAS), and Codex Alimentarius Commission (CAC) standards were used to interpret the results. The criteria used to interpret microbiological results are indicated in Table 1.

Statistical data analysis

A hierarchical cluster analysis with the furthest neighbour method and squared Euclidean distance (using SPSS Version 16.0 for Windows, SPSS Inc., Chicago, IL, USA) was used to analyse the hygienic performance data. A non-parametric test (Kruskal Wallis Non-Parametric H test) was performed to determine the differences among the clusters on the handling practices. Analysis of

microbiological data was by R statistical package (R Development Core Team Version 3.0, Vienna, Austria), whereby One-way ANOVA test was carried out to determine whether there are differences among the factors. Means were separated by Duncan Multiple Range Test (DMRT). The significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Hygienic performance of *Kayabo* processors

Characteristics of *Kayabo* processors

The hierarchical cluster analysis (average linkage) produced a dendrogram (Figure 1) showing three clusters. Cluster I had 15 processors from Kanyama, while cluster II composed of 10 processors and cluster III had 5 processors from Mwaloni. However, the processors ($n=15$) from Kanyama (Cluster I) were predominantly micro-scale, which were neither registered by the then Tanzania Food and Drugs Authority (TFDA), Municipal Council, nor by the Fisheries Department of the Ministry of Livestock and Fisheries (Table 2).

The majority ($20/30 = 66.67\%$) of *Kayabo* processors were women. These processors were micro- (36.67%) and small- (63.33%) scale with a weekly processing capacity of 1-4 tons. Previous studies found that domestic-oriented fish processing companies are mainly artisanal and of small-scale (Kussaga et al., 2013; Akintola and Fakoya, 2017) and dominated by women carrying out fish smoking, salting, and drying (Kweka et al., 2006; Akande and Diei-Ouadi, 2010). The current study revealed that all processors (100%) had primary level education; consequently, none of them had attended any training on food hygiene and processing (Table 2). This situation could lead to inadequate processing and handling of *Kayabo*. Likewise, other studies reported low levels of formal education among the fishers and processors (Omwega et al., 2006; Olale et al., 2010; Ibrahim et al., 2011; Akintola and Fakoya, 2017). It is, however, reported that trained personnel could use appropriate fish handling practices in value chain processes (Davies, 2009; Akande and Diei-Ouadi, 2010), which might reduce post-harvest losses and improve the quality of the products. All processors in clusters I and III had a weekly processing capacity of 1 ton, whereas those in Cluster II could process 4 tons per week (Table 2). Processors in Cluster I operated in unregistered premises with poorly designed facilities. They did operate at an informal level without any control/monitoring from the food safety control authorities or health officers. Similar studies in Nigeria reported inadequate contact of processors with the extension agents, which deny them access to improved processing techniques (Ibrahim et al., 2011). Products from such premises are often of low quality and unsafe due to possible contamination with food safety hazards (FAO/CDR, 2013).

Table 1. Microbiological criteria of fish and fishery products.

S/N	Parameter	Criteria (Maximum Limit)	Source
1	Total Viable Count (TVC)	1×10^5 CFU/g	TZS 118:2007- EAS 828:2016
2	<i>E. coli</i>	1×10^1 MPN/g Absent	TZS 731:2007 EAS 828:2016 CAC/RCP 52-2003
3	Total coliforms	4×10^2 MPN/g	TZS 119:2002
4	<i>S. aureus</i>	1×10^3 CFU/g 2×10^3 CFU/g	TZS 125:2002 EAS 828:2016
5	<i>L. monocytogenes</i>	Absent in 25g	CAC/RCP 52

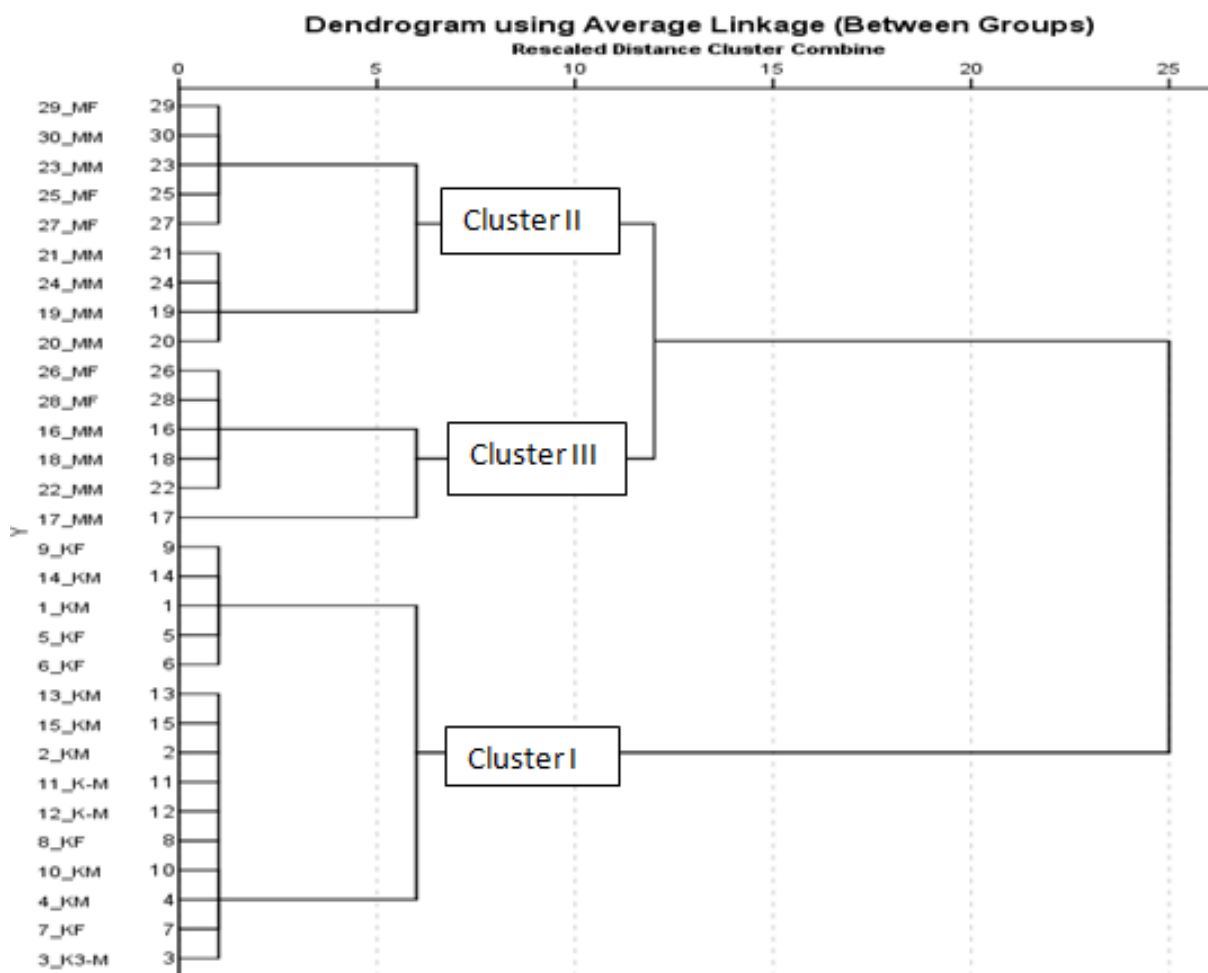


Figure 1. A dendrogram showing clusters by hierarchical cluster analysis on the relationship of *Kayaboprocessors* in Kanyama and Mwaloni according to their handling practices.

Site and building layout

Table 3 shows the site and building layout of *Kayabo* processors. It shows that all processors operated in

inadequately designed premises without good drainage systems. Particularly, 'Cluster I' processors had an unfenced processing premise to control access of pests, animals, and unauthorized people. Animals were roaming

Table 2. Characteristics of *Kayabo* processors (n=30).

Parameter	Category	Frequency (Percentage)		
		Cluster I	Cluster II	Cluster III
Gender	Male	5 (33.3)*	3 (30.0)	2 (40.0)
	Female	10 (67.7)	7 (70.0)	3 (60.0)
Education level	Informal	0 (0)	0 (0)	0 (0)
	Primary	15 (100)	10 (100)	5 (100)
	Secondary	0 (0)	0 (0)	0 (0)
Number of employees	<10 (micro)	6 (40.0)	2 (20.0)	3 (60.0)
	10-49 (small)	9 (60.0)	8 (80.0)	2 (40.0)
	50-100 (medium)	0 (0)	0 (0)	0 (0)
Production volume	1 ton/week	15 (100)	0(0)	5 (100)
	2 tons/week	0 (0)	0 (0)	0 (0)
	3 tons/week	0 (0)	0 (0)	0 (0)
	4 tons/week	0 (0)	10 (0)	0 (00)
Registration status	Not registered	15 (100)	0(0)	0 (0)
	Registered	0 (0)	10 (100)	5 (100)
	In process	0 (0)	0 (0)	0 (0)

*numbers in brackets are percentages.

around the processing premise. Although processors in clusters II and III had fenced premises, there was no control of food vendors (including fruits and vegetables) into the site. Animals and fruits and vegetables could be vehicles of microbiological hazards that could subsequently cross-contaminate the premises and products.

Moreover, the majority of processors in cluster I (100%), cluster III (80%), and cluster II (30%) had buildings not constructed with permanent materials. The buildings were makeshifts; constructed with wooden materials and thatched with plastic sheets or dried grasses without ceilings. The roofs could leak during rains to spoil or contaminate the products. Unfortunately, all processors used inadequately designed equipment and had no cold storage facilities. However, all processors used potable water from the Municipal supply (Table 3). Lack of adequate knowledge on proper food handling accompanied by poor design and layout of the premises (no separation between dirty and clean processes) could lead to cross-contamination. Previous studies reported inadequate facilities and the use of traditional tools and practices for fish processing (Ibrahim et al., 2011; Akintola and Fakoya, 2017). Such ill-practices are linked to low quality and unsafe products.

Sanitation and personnel hygiene

Table 4 shows the sanitation of premises and equipment and personal hygiene. It revealed that personnel were not

subjected to any medical examination as required by the Tanzanian food laws and regulations. Tanzanian food law requires that all food handlers check their health when employed and after every six months (TFDCA, 2003). In addition, it prohibits personnel suffering from a septic sore, diarrhoea, chronic cough or septic sore throat, typhoid, paratyphoid fever, any *Salmonella* infection, dysentery, or any staphylococcal infection to handle food (TFDCA, 2003).

Moreover, all processors had no hygienically designed toilets with hand-washing facilities. Although cluster I processors had toilets, there were pit latrines located several meters away from the processing building and without water supply. Besides, food handlers had never received any training on food processing and good hygienic (GHP) and manufacturing (GMP) practices. Eventually, none of the personnel had put on personal protective gears and uniforms to minimize (cross) contamination. Such unhygienic conditions could result in product contamination with both pathogenic and spoilage microorganisms. Previous studies also observed inadequate conditions by artisanal and small-scale fish processors (Ibrahim et al., 2011; Akintola and Fakoya, 2017). The majority of food-borne outbreaks occur as a result of the failure of the food preparation procedures to adhere to good hygienic practices (FAO/WHO, 2013). Fish is a perishable product that needs good hygienic practices along the value chain to control microbial contamination and spoilage (Okonkwo et al., 1993; Nguyen et al., 2007). Inadequate cleaning and sanitation of processing equipment like containers, knives, and

Table 3. Frequency distribution of *Kayabo* processors according to their site, building layout and sources of water.

Parameter	Attribute	Category	Frequency (Percentage)		
			Cluster I	Cluster II	Cluster III
Site/Location	Free from contamination	Yes	0 (0)	0 (0)	0 (0)
		No	15 (100)	10 (100)	5 (100)
		Total	15 (100)	10 (100)	5 (100)
	Sound water drainage	Yes	0 (0)	10 (100)	5 (100)
		No	15 (100)	0 (0)	0 (0)
		Total	15(100)	10 (100)	5 (100)
	Road accessibility	Yes	15 (100)	10 (100)	5 (100)
		No	0 (0)	0 (0)	0 (0)
		Total	15(100)	10(100)	5 (100)
Adequate area / space	Yes	0 (0)	0 (0)	0 (0)	
	No	15 (100)	10 (100)	5 (100)	
	Total	15 (100)	10 (100)	5 (100)	
Building layout /Equipment design	Permanent materials	Yes	0 (0)	7 (70.0)	1 (20.0)
		No	15 (100)	3 (30.0)	4 (80.0)
		Total	15 (100)	10 (100)	5 (100)
	Roofing	Yes	0 (0)	7 (70.0)	1 (20.0)
		No	15 (100)	3 (30.0)	4 (80.0)
		Total	15 (100)	10 (100)	5 (100)
	Food grade equipment	Yes	0 (0)	0 (0)	0 (0)
		No	15 (100)	10 (100)	5 (100)
		Total	15 (100)	10 (100)	5 (100)
Cold storage	Yes	0 (0)	0 (0)	0 (0)	
	No	15 (100)	15 (100)	5 (100)	
	Total	15 (100)	10 (100)	5 (100)	
Cross contamination	Yes	15(100)	10(100)	5 (100)	
	No	0(0)	0(0)	0 (0)	
	Total	15 (100)	10 (100)	5 (100)	
Water source	Municipal	Yes	15 (100)	10 (100)	5 (100)
		No	0 (0)	0 (0)	0 (0)
		Total	15 (100)	10 (100)	5 (100)

contact surfaces is a potential source of bacterial contamination in fish processing operations (Reij and Aantrekker, 2004; FAO/WHO, 2009) and could result into spoilage.

Source of raw materials, processing and packaging of final products

The majority of *Kayabo* processors (80% in cluster II, 67% in Cluster I, and 60% in Cluster III) obtained their

raw materials (*Lates niloticus*) as rejects from large fishprocessing factories, for the export market (Table 5). While raw materials and products are properly handled in such factories, rejects are not hygienically handled or chilled. Although perishable raw materials like fish would require chill storage (between -1 and 4°C) to minimize the growth of micro-organisms and spoilage (Berkel et al., 2004; Ghaly et al., 2010), none of the processors had cold storage facilities. This could result in the proliferation of microorganisms and toxins formation. However, washing of Nile perch prior to processing was done by all

Table 4. Frequency distribution of processors according to sanitation and personnel hygiene practices.

Requirement	Category	Frequency (percentage)		
		Cluster I	Cluster II	Cluster III
Medical examination	Yes	0 (0)	0 (0)	0 (0)
	No	15 (100)	10 (100)	5 (100)
	Total	15 (100)	10 (100)	5 (100)
Toilets	Yes	15 (100)	10 (100)	5 (100)
	No	0 (0)	0 (0)	0 (0)
	Total	15 (100)	10 (100)	5 (100)
Personal hygiene facilities	Toilet adequacy	Yes	0 (0)	0 (0)
	No	15 (100)	10 (100)	5 (100)
	Total	15 (100)	10 (100)	5 (100)
Toilet water supply	Yes	0 (0)	10 (100)	5 (100)
	No	15 (100)	0 (0)	0 (0)
	Total	15 (100)	10 (100)	5 (100)
Protective gears	Yes	0 (0)	0 (0)	0 (0)
	No	15 (100)	10 (100)	5 (100)
	Total	15 (100)	10 (100)	5 (100)
Training on hygiene	Yes	0 (0)	0 (0)	0 (0)
	No	15 (100)	10 (100)	5 (100)
	Total	15 (100)	10 (100)	5 (100)
Pre and post processing cleaning	Yes	0 (0)	0 (0)	0 (0)
	No	15 (100)	10 (100)	5 (100)
	Total	15 (100)	10 (100)	5 (100)

processors and fish curing was done by using iodized salt. Rough food contact surfaces of drying racks (often made of wooden materials) are difficult to clean and sanitize; they are harbourage areas of pests, pathogenic and spoilage micro-organisms.

Generally, *Kayabo* was packaged in bulk (25-30 kg). Bulky packaging was carried out with non-food grade materials such as re-used, non-cleaned, and sanitized polyethylene bags. The packaging was carried out in a very unhygienic and rudimentary condition. After filling the polythene bags by hand, the products were compressed by using bare feet. These ill-practices could expose the products to various microbiological hazards (FAO/WHO, 2009; FAO/CDR, 2013). To ensure the quality and safety of *Kayabo*, proper, and hygienic packaging practices are the pre-requisites (FAO/WHO, 2009).

Assessment of microbiological contamination

The TVC were quantified in all clusters with levels of

contamination ranging from 2.08 - 8.68 Log CFU/25 g (Table 6). While cluster I had 48% (12/15) of the processors exceeding the maximum allowable limit (5 Log CFU/25 g) for TVC; none of the processors from cluster II and cluster III exceeded the legal limit. This current study reported high TVC than a study in salted sun-dried Nile perch products which observed 2.9-4.5 log CFU/g (Baniga et al., 2019). *Staphylococcus aureus* counts ranging from <1 to 5.49 Log CFU/25 g were quantified from 47% (14/30) of processors; however, majority (12/14) were from Cluster I (Table 6). About 42% (5/12) of processors from Cluster I exceeded the allowable limit (3 Log CFU/25 g) for *S. aureus*. Although total coliforms were occasionally quantified in Cluster I (2/15 processors) and Cluster II (1/10 processors), they were within the legal limits. Unexpectedly, pathogens were either below the quantification (<1 Log CFU/25 g for *Escherichia coli*) or not detected (Absent, P/25 g for *L. monocytogenes*) throughout the study.

The counts beyond the acceptable limits for TVC (5.14 - 8.68 Log CFU/25 g) and *S. aureus* (3.08- 5.49 Log CFU/25 g) of the analysed samples from the majority of

Table 5. Frequency distribution (and percentages) of processors according to the source of raw materials, processing and packaging methods.

Parameter		Category	Cluster I	Cluster II	Cluster III	
Raw materials (Nile perch)	Specified sources	Rejects from fish factories	10 (66.7)	8 (80.0)	3 (60.0)	
		Artisanal fishermen	2 (13.3)	1 (10.0)	2 (40.0)	
		Rejects from landing sites	3 (20.0)	1 (10.0)	0 (0)	
		Total	15 (100)	10 (100)	5 (100)	
	Cold storage from the source	Yes	0 (0)	8 (80.0)	4 (80.0)	
		No	15 (100)	2 (20.0)	1 (20.0)	
		Total	15 (100)	10 (100)	5 (100)	
	On site cold storage	Yes	0 (0)	0 (0)	0 (0)	
		No	15 (100)	10 (100)	5 (100)	
		Total	15 (100)	10 (100)	5 (100)	
	Raw fish washing prior to processing	Yes	15 (100)	10 (100)	5 (100)	
		No	0 (0)	0 (0)	0 (0)	
		Total	15 (100)	10 (100)	5 (100)	
	Type of salt used	Iodated	15 (100)	10 (100)	5 (100)	
		Rocky	0 (0)	0 (0)	0 (0)	
		Total	15 (100)	10 (100)	5 (100)	
	Processing	Food grade Equipment	In place	0 (0)	0 (0)	0 (0)
			Not in place	15 (100)	10 (100)	5 (100)
			Total	15 (100)	10 (100)	5 (100)
	Product packaging	Yes	10 (66.7)	7 (70.0)	4 (80)	
No		5 (33.3)	3 (30.0)	1 (20)		
Total		15 (100)	10 (100)	5 (100)		
Type of packaging	Food grade	0 (0)	0 (0)	0 (0)		
	Non-food grade	15 (100)	15 (100)	5 (100)		
	Total	15 (100)	10 (100)	5 (100)		
Labeling	Yes	0 (0)	0 (0)	0 (0)		
	No	15 (100)	10 (100)	5 (100)		
	Total	15 (100)	10 (100)	5 (100)		

processors in Cluster I are due to the observed unhygienic handling conditions of raw materials and finished products, poor sanitation and personal hygiene, use of non-food grade equipment (wooden materials as drying racks which are difficult to clean and sanitize hence harbouring microorganisms) and inadequate packaging conditions. According to Norman (1999) and Kussaga et al. (2017), possible causes of food contamination by enteric microorganisms (such as, *Enterobacteriaceae* and *E. coli*) are inadequate procedures

of handling, packaging, storage, sanitation, and personal hygiene.

Despite the observed poor hygienic conditions, all *Kayabo* samples tested for total coliforms, *E. coli* and *L. monocytogenes* complied with the legal limits. This could be due to the effect of salt (Sodium chloride) on water activity (*aw*) as well as moisture content of *Kayabo*, which inhibits the growth and proliferation of pathogenic microorganisms. A similar study by Wijnker et al. (2006) on antimicrobial properties of salt (NaCl) in fish, fishery

Table 6. Level of microbiological contamination of *Kayabof* from all processors.

Cluster	Processor	TVC	S. aureus	Total coliforms
		Log (CFU/25 g)	Log (CFU/25 g)	Log (MPN/25 g)
I	K01	8.68 ^a	2.61 ⁱ	**
	K02	5.41 ⁱ	3.26 ^e	**
	K03	4.11 ⁿ	3.08 ^g	0.60 ^b
	K04	6.20 ^f	3.43 ^d	1.36 ^a
	K05	5.14 ^k	2.36 ^k	**
	K06	6.78 ^e	3.18 ^f	**
	K07	7.40 ^d	*	**
	K08	5.20 ^j	2.56 ^j	**
	K09	7.86 ^b	5.08 ^b	**
	K10	7.61 ^c	5.49 ^a	**
	K11	7.40 ^d	4.28 ^c	**
	K12	5.48 ^h	*	**
	K13	4.90 ^l	2.36 ^k	**
	K14	4.54 ^m	*	**
	K15	5.94 ^g	2.86 ^h	**
II	M01	3.65 ^c	*	**
	M02	2.85 ^g	*	**
	M03	3.20 ^f	*	**
	M04	3.51 ^d	*	0.6
	M05	4.82 ^a	2.65	**
	M06	3.81 ^b	*	**
	M07	2.11 ⁱ	*	**
	M09	2.34 ^h	*	**
	M11	2.08 ^j	*	**
	M13	3.41 ^e	*	**
	M08	2.59 ^d	*	**
	M10	4.63 ^a	*	**
III	M12	3.66 ^b	*	**
	M14	3.26 ^c	2.36	**
	M15	2.18 ^e	*	**
MAL		5	3	2

MAL means Maximum Allowable Limit (ISO; 4833:2003, 6888 – 3: 2003, 4831:2006, respectively). K stands for Kanyama location and M for Mwaloni location.

*Microbial count < 1.0 x 10¹ log (CFU/g), **Microbial count < 0.3 x 10¹ log (MPN/g)

products, and natural sheep casings at different water activity levels found that microbiological (*E. coli*, *Salmonella typhimurium*, *L. monocytogenes*, *S. aureus* and *E. coli* O157: H7) activities stopped when water activity of 0.89 was reached. The inhibitory effects of sucrose and sodium chloride on *S. aureus* are primarily related to lower water activity (Dave and Ghaly, 2011). Sodium chloride has more inhibitory effects than glycerol in inhibiting the growth of spoilage microorganisms. Salting decreases water activity and has an inhibitory effect on pathogenic bacteria (Teklemariam et al., 2015).

The TVC variation was significantly different (P<0.05)

among the clusters. Although coliforms and *S. aureus* were observed in all companies among the clusters, the variations were not significantly different. Post Hoc (Bonferroni) test indicated that a significant difference (P<0.05) in TVC between Clusters I and II and Clusters I and III (Table 7). As compared with other clusters, 'cluster I' had significantly high contamination with TVC (6.17 Log CFU/25 g) and *S. aureus* (3.38 Log CFU/25 g). The level of TVC and *S. aureus* was beyond the respective legal limits. Microbiological assessment results correspond with the prevailing conditions as cluster I had an unfenced processing site to control pests, animals, and

Table 7. Mean values of TVC, total coliforms and *S. aureus* from cluster I, II and III.

Parameter	Mean values		
	Cluster I	Cluster II	Cluster III
TVC	6.17 ^a	3.18 ^b	3.26 ^b
<i>S. aureus</i>	3.38 ^a	2.65 ^a	2.36 ^a
Total coliforms	0.98 ^a	0.60 ^a	0.00 ^a

Means with different superscripts in the same row are significant different at P<0.05.

unauthorized people, as well as animals, could access the premises.

Conclusions

Kayabo processing is dominated by women, often without the knowledge of proper food handling and operating in very unhygienic conditions. The raw materials are of inadequate quality, lack of food-grade processing equipment, and poor packaging and storage conditions. Although the processors receive inadequate handled raw materials (as rejects) from fish processing companies accompanied by poor processing and storage conditions, some processors had products with less microbiological contamination. This could be attributed to the use of the curing process, which inhibits growth and possibly kill micro-organisms. If these processors are trained on best handling practices and receive quality raw materials, they could process products of good microbial quality. Moreover, fish companies for the domestic market did not receive the same level of control as for the export. Therefore, similar level of control in the domestic oriented companies is highly recommended.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

- Abila RO (2000). Development of the Lake Victoria Fishery: A Boon or Bane for Food Security? Socio-economics of the Lake Victoria Fisheries Project Technical Report No. 7. IUCN-EARO, Nairobi 28 p.
- Akande G, Diei-Ouadi Y (2010). Post-harvest Losses in Small-scale Fisheries: Case Studies in Five Sub-Saharan African Countries. Fisheries and Aquaculture Technical Paper. No. 550. FAO, Rome 72 p.
- Akintola SL, Fakoya KA (2017). Small-scale fisheries in the context of traditional post-harvest practice and the quest for food and nutritional security in Nigeria. *Agriculture and Food Security* 6(1):34.
- Amos T (2007). Production and productivity of crustaceans in Nigeria. *Journal of Social Science* 15:229-233.
- Baniga Z, Dalsgaard A, Kusiluka LJM, Mdegela RH (2019). Microbial quality of Nile perch (*Lates niloticus*) and physico-chemical properties of salted sun-dried products sold at regional markets, Tanzania. *African Journal of Microbiology Research* 13(7):128-133.
- Berkel BM, van Boogaard B, Heijnen C (2004). Preservation of Fish and Meat. Digigrafi, Wageningen, the Netherlands P 86.
- Berthe T, Ratajczak M, Clermont O, Denamur E, Petit F (2013). Evidence for coexistence of distinct *Escherichia coli* populations in various aquatic environments and their survival in estuary water. *Applied Environmental Microbiology* 79(15):4684-4693.
- Dave D, Ghaly AE (2011). Meat spoilage mechanisms and preservation techniques: A critical review. *American Journal of Agriculture and Biological Sciences* 6(4):486-510.
- Davies RM (2009). Traditional and improved fish processing technologies in Bayelsa State, Nigeria. *European Journal of Scientific Research* 26(4):539-548.
- El-Sherif SAEH, Abd El-Ghafour S (2016). Investigation of the quality properties and nutritional values of four fish species from lake qaroun, Egypt. *International Journal of Chemical and Technical Research* 9(4):16-26.
- FAO (2014). Present and future markets for fish and fish products from small-scale fisheries - case studies from Asia, Africa and Latin America. FAO Report (Vol. 1033).
- FAO/Corporate Document Repository, FAO/CDR (2013). Assurance of seafood quality Fisheries and Aquaculture Department. Available at: <http://www.fao.org/docrep/003/t1768e/t1768e04.htm>.
- FAO/WHO (2009). Code of practice for fish and fishery products. Rome, Italy. Available at: <http://www.fao.org/3/a1553e/a1553e00.htm>
- Ghaly AE, Dave D, Budge S, Brooks MS (2010). Fish spoilage mechanisms and preservation techniques. *American Journal of Applied Science* 7(7):859-877.
- Ibrahim HI, Kigbu AA, Mohammed R (2011). Women's experiences in small scale fish processing in Lake Feferuwa fishing community, Nasarawa State, Nigeria. *Livestock Research for Rural Development* 23(3):1-8.
- ISO (2003a). Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of microorganisms - Colony-count technique at 30 °C. 9 p. Available at: <https://www.iso.org/standard/34524.html>.
- ISO (2003b). Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase - positive staphylococci (*Staphylococcus aureus* and other species) - Part 1: Technique using Baird-Parker agar medium- Amendment 1: Inclusion of precision data 11 p.
- ISO (2004). Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method 16 p.
- ISO (2005). Microbiology of food and animal feeding stuffs- Horizontal Method for the detection and enumeration of presumptive *E. coli* - Most probable number technique. Available at: <https://www.iso.org/standard/34568.html>
- ISO (2006). Microbiology of food and animal feeding stuffs - Horizontal Method for the detection and enumeration of coliforms - Most probable number technique. Available at: <https://www.iso.org/standard/38280.html>

- Jacxsens L, Kussaga J, Luning PA, Van der Spiegel M, Uyttendaele M (2009). A microbial assessment scheme to support microbial performance measurements of food safety management systems. *International Journal of Food Microbiology* 134:113-125.
- Jinadasa BK (2014). Determination of Quality of Marine Fishes Based on Total Volatile Base Nitrogen test (TVB-N). *Natural Science* 12(5):106-111.
- Kabahenda MK, Omony P, Hüsken SM (2009). Post-harvest handling of low-value fish products and threats to nutritional quality: a review of practices in the Lake Victoria region. Available at: https://digitalarchive.worldfishcenter.org/bitstream/handle/20.500.123.48/1463/WF_3373.pdf?sequence=1&isAllowed=y
- Kussaga JB, Jacxsens L, Luning PA, Tiisekwa BPM (2013). Diagnosis of food safety management systems performance in food processing sectors for export and domestic markets. *African Journal of Food Science and Technology* 4(10):240-250.
- Kussaga JB, Luning PA, Tiisekwa BPM, Jacxsens L (2014). Challenges in Performance of Food Safety Management Systems: a case of Fish Processing Companies in Tanzania. *Journal of Food Protection*, 77(4):621-630.
- Kussaga JB, Luning PA, Tiisekwa BPM, Jacxsens L (2017). Microbiological performance of HACCP-based food safety management systems: a case of Nile perch processing company. *African Journal of Food Science* 11(7):200-214.
- Kweka J, Musa J, Kabelwa G (2006). The Linkages Between Trade, Development And Poverty Reduction: The case study of fisheries sub-sector in Tanzania. *Economic and Social Research Foundation* pp. 1-19.
- LVFO (2009). The fisheries management plan for Lake Victoria 2009–2014.
- Medard M, van Dijk H, Hebinck P (2019). Competing for kayabo: gendered struggles for fish and livelihood on the shore of Lake Victoria. *Maritime Studies* 18:321-333.
- Nguyen TL, Dalsgaard A, Phung DC, Mara D (2007). Microbiological quality of fish grown in wastewater-fed and non-wastewater-fed fish ponds in Hanoi, Vietnam: influence of hygiene practices in local retail markets. *Journal of Water Health* 5(2):209-218.
- Norman GM (1999). *Principles of Food Sanitation; Fourth Edition*. Aspen Publishers, Inc. 253 p.
- Okonkwo TM, Obanu ZA, Oludusin AO (1993). The safety traditionally smoked fish products with respect to *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus flavus*. *Proceeding of FAO expert Consultation on Fish Technology in Africa, Accra, Ghana, 22-25 October, 1991*. pp. 210-227.
- Olale E, Henson S, Cranfield J (2010). Determinants of income diversification among fishing communities in Western Kenya. Selected paper prepared for presentation at the Agricultural and applied economics association 2010. Joint Annual Meeting, Denver, Colorado 31 p.
- Omwega RN, Abila R, Lwenya C (2006). Fishing and poverty levels around Lake Victoria (Kenya). *Proceedings of the 11th World Lakes Conference, Lake Victoria* pp. 193-199.
- Quang NH (2005). Guidelines for Handling and Preservation of Fresh Fish for Further Processing in Vietnam. *United Nations Universities Fisheries Programme* 57 p.
- Reij MW, Aantrekker ED (2004). Recontamination as a source of pathogens in processed foods. *International Journal of Food Microbiology* 91(1):1-11.
- Teklemariam AD, Tessema F, Abayneh T (2015). Review on evaluation of safety of fish and fish products. *International Journal of Fisheries and Aquatic Studies* 3(2):111-117.
- TFDCA (2003). Tanzania Food, Drugs and Cosmetics Act. Available at: <https://tanzania.eregulations.org/media/TFDA%20ACT.pdf>
- United Republic of Tanzania (URT) (2013). Fisheries Sector Development Programme: Fisheries sector Development Programme. *Livestock and Fisheries Development* pp. 25-27.
- US-FDA BAM (2001). Manual: Bacteriological Analytical Manual. Available at: <https://www.epa.gov/sites/production/files/2015-07/documents/fda-bam-appendix1.pdf>
- Wijnker JJ, Koop G, Lipman LJA (2006). Antimicrobial properties of salt (NaCl) used for the preservation of natural casings. *Food Microbiology* 23:657-662.

Full Length Research Paper

Quality and sensory properties of instant fried noodles made with soybean and carrot pomace flour

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Wheat flour commonly used in making noodles is rich in starch but poor in protein and fiber. Wheat flour substituted with soybean and carrot pomace flours were used to produce instant fried noodles. Soybean is high in protein while carrot pomace is rich in dietary fiber. The aim of this study was to evaluate the effect of substituting wheat flour with soybean and carrot pomace flour on the physicochemical, cooking and sensory properties of instant fried noodles. Four flour blends in ratios of 100:0:0, 80:15:5, 70:20:10, 60:25:15 wheat, soybean and carrot pomace flour respectively were prepared. The results indicated a significant difference ($P < 0.05$) in protein and crude fiber content of the noodles made from the flour blends. The cooking loss and water absorption increased with increase in the amount of substituted soybean and carrot pomace flour. There was no significant difference in the tensile strength among noodles. However, breaking length of the noodles decreased with increase in replacement of soybean and carrot pomace flour. The noodles decreased in brightness with increased carrot pomace substitution. Noodles made from 80% wheat flour, 15% soybean flour and 5% carrot pomace were the most preferred by the sensory panelist. Incorporation of soybean flour and carrot pomace flour improved the nutritional quality and sensory attributes of the instant fried noodles.

Key words: Instant fried noodles, carrot pomace flour, soybean flour.

INTRODUCTION

Consumption of noodles has increased worldwide because of their convenience, palatability, shelf stability and affordability. Noodles are pasta products mainly made from flour, water, salt. The properties of the flour, type of salt and the manufacturing process leads to a wide array of noodles types (Fu, 2008; Gulia et al., 2014). Noodles are mainly made by a process of mixing raw materials, dough sheeting, compounding, sheeting / rolling and cutting. Noodle strands coming out of cutting

rolls are further processed to produce different types of noodles (Fu, 2008; Gulia et al., 2014; Adejuwon et al., 2020). Two types of noodles are generally known, white salted noodles made from flour, sodium chloride and water, and yellow alkaline noodles made from flour, alkaline salts (sodium tripolyphosphate and sodium carbonate) and water (Asenstorfer et al., 2006; Siah and Quail, 2018).

Wheat flour used in making noodles is rich in starch but

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low in protein (essential amino acids especially lysine), dietary fiber and vitamins which are lost during wheat flour refinement (Dewettinck et al., 2008). Previous studies have tried solving this problem by substituting wheat flour with various foods rich in protein and fiber. These includes substituting wheat flour with potato flour (Kang et al., 2017), soy flour (Rani et al., 2019), seaweed flour (Kumoro et al., 2016), lentil (Rathod and Annapure, 2017), ground linseed (Zhu and Li, 2019), apple pomace (Xu et al., 2020), carrot puree (Prerana and Anupama, 2020), finger millet (Hymavathi et al., 2019) and oyster mushrooms (Arora et al., 2018). Limited studies have incorporated soybean flour together with carrot pomace in the noodles. Soybean flour provides extra protein while carrot pomace provides fiber and beta carotene to the wheat flour noodles.

Soybeans are excellent sources of protein (35-40%), rich in calcium, iron, phosphorous and vitamins (Udachan et al., 2018). Noodles made mainly from wheat flour are rich in carbohydrates but are deficient in terms of protein quality and amino acid (Udachan et al., 2018). Incorporation of soybean flour seeks to improve the protein quality of noodles. Several studies have successfully incorporated soy flour in noodles (Pakhare et al., 2016; Jalgaonkar et al., 2018; Huh et al., 2019; Rani et al., 2019, 2020; Violalita et al., 2020). Pakhare et al. (2016) reported a significant increase in protein content of noodles supplemented with defatted soy bean flour. Cooking loss and cooking time of the noodles also increased significantly.

Carrot has gained increased attention over the years due to its richness in antioxidants and beta carotene (pro-vitamin A) (Sharma et al., 2012). Carrot pomace is the by-product of carrot juice extraction unit. During carrot juice production, 30-50% of the carrot remains as pomace, and up to 50% of the carotene is lost with the pomace (Sharma et al., 2012). The pomace is mainly disposed as feed or manure, however, it contains a good residual amount of all the vitamins, minerals and dietary fiber (Surbhi et al., 2018). Incorporation of carrot pomace in food will decrease its environmental load problem. Various attempts have been made at utilizing carrot pomace in foods such as cookies (Ahmad et al., 2016; Sahni and Shere, 2017), cake (Majzoobi et al., 2016; Semwal et al., 2016), sausage (Yadav et al., 2018) and pasta (Jalgaonkar et al., 2018).

The aim of this study was to evaluate the effect of incorporating different proportions of soybean flour and carrot pomace flour on nutritional composition, physical properties and sensory characteristics of instant fried noodles.

MATERIALS AND METHODS

Wheat and soybean flour

Commercial wheat flour was obtained from a local supermarket, defatted soy flour was obtained from Soy Africa Company, Kenya.

Preparation of carrot pomace flour

Fresh carrots were purchased from a local market in Juja, Kenya. Pomace flour was obtained according to a method described by Gull et al. (2015). The carrots were washed in running tap water to remove extraneous material followed by juice extraction. Carrot pomace was collected, spread on aluminum foils and oven dried at 60°C for 4 h. The dried pomace was ground and packed in air tight container for further use.

Formulation of composite flour

Wheat flour, soybean flour and carrot pomace flour were mixed at different proportions as shown in Table 1.

Noodle production

The noodles were processed at Jomo Kenyatta University of Agriculture and Technology (JKUAT) Nissin Foods Limited. Recipe for making noodles was provided by JKUAT Nissin Foods. One kilogram of each flour blends (as shown in Table 1) were mixed separately with water (360 ml/kg), table salt (16 g/kg), sodium bicarbonate (2 g/kg) and sodium tri polyphosphate (2 g/kg) in a rotary mixer. A thick sheet of dough formed which was allowed to rest for 20 min in a plastic bag. The dough was then passed through extension rollers to reduce thickness. The thin sheet of dough produced was passed through a shredder. The shredded dough was then cut at 10 cm spacing, steamed for 20 min and deep fried at 140°C in palm oil for 30 s. The instant fried noodles were then cooled at room temperature and packed in airtight bags.

Chemical analysis

Proximate analysis

Proximate analysis of instant fried noodles prepared from the different flour blends were determined using standard methods of AOAC (2010). Proximate analysis was done in triplicate to obtain a mean value for each nutrient.

Total carotenoid content

This was determined using AOAC (2010) method. About 2 g of each sample was weighed, transferred into a mortar and a 10 ml of acetone added. A pinch of acid washed sand was added and the sample was thoroughly ground using pestle until it produced no more color. The acetone extract was transferred into a 25 ml volumetric flask and topped up with acetone. The extract was transferred into a separating column and 25 ml of petroleum ether was added. The separating column was shaken vigorously then allowed to stand. The mixture separated into two, and acetone which is at the bottom was released from the column. Distilled water was added to wash off the acetone, this was done severally to completely wash off the acetone. After extraction, the total carotenoid content was determined using UV-Vis spectrophotometer (PD 3000UV APEL Co.) at 450 nm. Beta carotene standard curve was used to calculate the total carotenoid content. Results were presented as mg/100 g.

Mineral content analysis

Mineral content (calcium (Ca), magnesium (Mg), zinc (Zn) and phosphorous (P)) of the noodles were determined using atomic

Table 1. Quantity of wheat, soybean and carrot pomace flour blends prepared.

Flour blends	Wheat flour (%)	Soybean flour (%)	Carrot pomace (%)
Control	100	0	0
Sample A	80	15	5
Sample B	70	20	10
Sample C	60	25	15

absorption spectroscopy (AAS) (model 210 VPG) according to AOAC (2010) method. Absorbance was recorded and standard curves of each individual element were plotted. Results were expressed as mg/100 g sample.

Carbohydrate content

The difference method was used to determine carbohydrate content of the noodles as follows:

% Carbohydrate = 100 - (% moisture content + % crude fibre + % ash content + % fat content + % protein).

Physical measurements

Cooking time

This was determined by noticing the time of disappearance of the core of the noodle strand during cooking (every 30 s) by squeezing the noodles between two transparent glasses slides (Prerana and Anupama, 2020).

Cooking loss

This is the amount of solid substance lost into the cooking water and was determined according to the method described by Ritthiruangdej et al. (2011). Ten grams of the sample noodles was placed in 300 ml of boiling distilled water in a 500 ml beaker and cooked to optimum time. The cooking water was collected (thoroughly agitating the total cooking water before sampling) after draining the noodles. The cooking water was placed in aluminum vessel then placed in an oven at 105°C and evaporated to dryness. The residue was weighed and reported as percentage of the uncooked noodles.

$$\text{Cooking loss} = \frac{\text{Weight of remaining solid content after oven drying}}{\text{Weight of fresh noodles}} \times 100$$

Water absorption

Three grams of the sample noodles were boiled in 200 ml of water until completely cooked (when center of the noodles becoming transparent). The cooked noodles were washed with distilled water then drained for 5 min and weighed immediately. Water absorption was reported as percent increase in the weight of cooked noodles compared to the uncooked noodles weight (Takahashi et al., 2005).

$$\text{Water absorption} = \frac{(\text{Weight of cooked noodles} - \text{Weight of uncooked noodles})}{\text{Weight of uncooked noodles}} \times 100$$

Color analysis

The color of uncooked noodles samples were measured using a Hunter Lab color analyzer. In the Hunter's lab colorimeter, the color of a sample is denoted by the three dimensions, L*, a* and b*. The L* value is a measurement of brightness (100 for perfect white to 0 for black, as the eye would evaluate it), a* value represents the red-green coordinates (negative is green while positive is red), b* value is the blue to yellow coordinates (negative is blue while positive is yellow). The L*, a* and b* readings of the noodles were obtained directly from the instrument.

Noodle texture analysis

Texture analysis of cooked noodles was measured using a Texture Analyzer (TA-XT2i) under optimal test conditions. Measurements were carried out at room temperature exactly ten minutes after cooking according to the method described by Lu et al. (2009). Instrument was set at break mode: test speed 1 mm/s. Two texture parameters were obtained tensile strength (maximum force; N) and breaking length (distance at maximum force).

Sensory evaluation

All noodles samples were cooked to the optimum cooking time and served to 20 untrained panelist. Samples were coded with three-digit random numbers and served in random order. Parameters evaluated were aroma, taste, color, texture and overall acceptability using a 9 point hedonic scale, where 9 = like extremely and 1 = dislike extremely (Gunathilake and Abeyrathne, 2008).

Statistical analysis

The means and standard deviations were determined for all the nutritional, textural, cooking and sensory qualities studied. The significant differences of mean values were assessed with one-way analysis of variance (ANOVA) followed by Duncan's test using SPSS software (Version 17.0 Inc.). Significant differences were determined at P<0.05.

RESULTS AND DISCUSSION

Proximate analysis

Chemical composition of instant fried noodles with supplemented soybean flour and carrot pomace flour are shown in Table 2. Moisture content of the noodles decreased with increased supplementation with the control having the highest moisture content of 4.861%.

Table 2. Proximate composition of instant fried noodles supplemented with soybean flour and carrot pomace flour.

Sample	Carbohydrate (%)	Moisture (%)	Ash (%)	Crude fat (%)	Crude fiber (%)	Protein (%)	Total carotenoid (mg/100 g)
Control	81.085±0.102 ^a	4.861±0.424 ^a	0.592±0.044 ^c	4.235±0.007 ^a	0.913±0.071 ^d	8.314±0.071 ^d	ND
Sample A	79.214±0.082 ^b	4.497±0.401 ^b	1.521±0.052 ^b	3.161±0.125 ^b	1.446±0.132 ^c	10.161±1.701 ^c	1.336±0.528 ^c
Sample B	74.214±0.132 ^c	4.219±0.401 ^c	2.041±0.032 ^b	3.154±0.121 ^{ab}	2.103±0.076 ^b	14.311±2.436 ^b	3.592±0.494 ^b
Sample C	71.911±0.201 ^d	3.944±0.792 ^d	2.352±0.210 ^a	2.930 ±0.005 ^c	2.403±0.018 ^a	16.460±0.654 ^a	4.080±0.315 ^a

Control=100% wheat flour, Sample A=80% wheat flour, 15% soybean flour, 5% carrot pomace flour, Sample B = 70% wheat flour, 20% soybean flour, 10% carrot pomace flour, Sample C= 65% wheat flour, 25% soybean flour, 15% carrot pomace flour. Values shown as mean± standard deviation of triplicate analysis. Different superscript letters in a column indicate significant differences ($P \leq 0.05$). ND-Not detected.

Moisture content of the noodle strands to evaporate (Gulia et al., 2014; Adejuwon et al., 2020). Moisture content is critical in controlling microbial growth and hence determining the shelf life of the noodles. Carbohydrate content of the noodles decreased with substitution of wheat flour. Sample C with 25% soybean flour and 15% carrot pomace flour had the lowest carbohydrate content of 71.911%. Ash content increased with replacement of wheat flour, noodles containing 60% wheat flour (Sample C) had the highest ash content (2.352%) while the control had the lowest (0.592%). Ash content gives an indication of the availability of minerals in a given food sample. The increase in ash content is attributed to the added soybean and carrot pomace flour.

The percentage protein content of the instant noodles ranged from 8.314 to 16.46%. Sample C had the highest protein content while the control had the lowest. This is attributed to the increase in the proportion of soybean flour added which is rich in protein (Medic et al., 2014). This increase in protein is in agreement with previous studies (Collins and Pangloli, 1997; Adegunwa et al., 2012; Omeire et al., 2014; Rani et al., 2019).

There was a decrease in fat content with substitution of wheat flour, Sample C had the lowest content and was significantly different from other samples. The decrease could be due to

increase in the proportion of soy flour (defatted) in the flour blend. Collins and Pangloli (1997) reported a decrease in fat content of noodles with addition of defatted soybean flour, however, other studies observed an increment in fat content (Adegunwa et al., 2012; Omeire et al., 2014). The increase in the amount of carrot pomace substituted could have also led to decrease in the amount of oil absorbed by the noodles. In a previous study, preharvest-dropped apple powder (PDAP) appeared to be very effective in lowering oil absorption of instant fried noodles (Kim et al., 2013).

The highest fiber content was found in Sample C (2.403%) and the lowest value was in the control sample (0.913%). The increase in fiber content is due to increase in carrot pomace in the blended flour. Similar trends on increase in fiber content were also reported in biscuits with added carrot pomace (Baljeet et al., 2014; Parveen et al., 2017). Dietary fiber plays an important role in prevention of constipation, regulation of blood sugar, protection against heart diseases and prevention of certain forms of cancers (Rajpurohit, 2018).

Carrots is a rich source of carotenoids and beta carotene, they are precursor of vitamin A and act as free-radical scavengers (Sharma et al., 2012; Surbhi et al., 2018). Carotenoids have been linked

with the enhancement of immune system and decreased risk of degenerative diseases such as cancer, cardiovascular disease, age related macular degeneration and cataract formation (Faulks and Southon, 2001; Sharma et al., 2012). There was an increase in the total carotenoids content with increased carrot pomace supplementation as shown in Table 2.

Increase in total carotenoid content with replacement of wheat flour with carrot puree in noodles was also reported by Prerana and Anupama (2020). Since carotenoids are affected by heat (Rodriguez-Amaya and Kimura, 2004), this analysis was done immediately after production of the noodles. There was a significant difference in total carotenoid content of the noodles prepared from the flour blends, with Sample C having the highest amount.

Mineral concentration

Carrots are good sources of calcium and phosphorous (Surbhi et al., 2018) while soybeans are a good source of magnesium and zinc (Reddy and Duke, 2015). Substitution of wheat flour with carrot pomace and soybean flour led to an increase in the mineral concentration of dry instant noodles (Table 3). Sample C had the highest

Table 3. Mineral concentration (mg/100 g) of instant fried noodles supplemented with carrot pomace flour and soybean flours.

Sample	Zinc	Calcium	Magnesium	Phosphorous
Control	1.660±0.329 ^c	33.473±0.513 ^d	34.901± 3.510 ^d	3.31±0.011 ^c
Sample A	2.119±0.168 ^b	51.37±1.512 ^c	49.365± 1.3364 ^c	6.696±0.227 ^b
Sample B	2.451± 0.021 ^a	60.57± 1.70 ^b	53.651± 3.8395 ^b	6.994±2.991 ^b
Sample C	2.602± 0.034 ^a	68.98± 1.661 ^a	55.015± 1.9445 ^a	8.112± 0.1357 ^a

Control= 100% wheat, Sample A=80% wheat flour, 15% soybean flour, 5% carrot pomace flour, Sample B = 70% wheat flour, 20% soybean flour, 10% carrot pomace flour, Sample C= 65% wheat flour, 25% soybean flour, 15% carrot pomace flour. Values are shown as mean± standard deviation of triplicate analysis. Different superscript letters in a column indicate significant differences ($p \leq 0.05$).

Table 4. Cooking time and cooking loss of instant fried noodles supplemented with soybean flour and carrot pomace flour.

Sample	Optimum cooking time(min)	Cooking loss (%)	Water absorption (%)
Control	13.0	9.261±1.258 ^d	125.301±0.134 ^d
Sample A	13.5	9.353±0.124 ^c	128.103±1.534 ^c
Sample B	13.5	11.318±0.371 ^b	130.90± 0.673 ^b
Sample C	14.5	14.034±1.319 ^a	135.50± 0.321 ^a

Control= 100% wheat, Sample A=80% wheat flour, 15% soybean flour, 5% carrot pomace flour, Sample B = 70% wheat flour, 20% soybean flour, 10% carrot pomace flour, Sample C= 65% wheat flour, 25% soybean flour, 15% carrot pomace flour. Values are shown as mean± standard deviation of triplicate analysis. Different superscript letters in a column indicate significant differences ($P \leq 0.05$).

amount of zinc but was not significantly different from Sample B. Phosphorous content of Sample A and B was not significantly different. Prerana and Anupama (2020) also reported an increase in mineral content of noodles substituted with carrot puree.

Cooking qualities

Cooking time refers to the time in minutes required to gelatinize the starch core of noodles (Prerana and Anupama, 2020). The cooking time increase with replacement of wheat flour as shown in Table 4. Sample C cooked for 14.5 min, Sample A and B both took 13.5 min while the control cooked for 13 min. The cooking time of noodles in this study was higher than what was reported by Adegunwa et al. (2012); Jalgaonkar et al. (2018) and Rani et al. (2019). Prerana and Anupama (2020) reported a decrease in cooking time with replacement of wheat flour with carrot puree in noodles.

Cooking loss increased with replacement of wheat flour with soy flour and carrot pomace flour. Sample C had the highest cooking loss (Table 4). Cooking loss is an important factor denoting cooking quality of noodles. Structural stability of noodles during cooking process is determined by cooking loss. High cooking loss is undesirable because it represents high solubility of starch, resulting in turbid cooking water, low cooking

tolerance and sticky texture (Wandee et al., 2014). There was significant difference ($P < 0.05$) in the water absorption of all the noodles made. Water absorption increased with substitution of wheat flour. Sample C had the highest percent water absorption (135%) while the control had the lowest value of 125%. The increase in water absorption is attributed to the high protein content of soybean flour (Rani et al., 2019) and high fiber content in the carrot pomace flour.

Color characteristics of noodles

Color is an important quality trait in noodles. Noodles should be bright in color and slow in discoloration with time after processing (Fu, 2008). Red or dull grey colored noodles are undesirable to most consumers. Factors influencing the color of a product are alkaline formulations used during processing, flour refinement and enzymatic browning associated with polyphenol oxidase (Asenstorfer et al., 2006). Figure 1 shows noodles made from the different composite flour and control.

Color evaluation showed variation in the lightness value among the noodle samples as shown by the L values (Table 5). Lightness of the noodles decreased with replacement of wheat with soybean and carrot pomace flour. The redness (a^*) values increased with supplementation with Sample C having the highest value

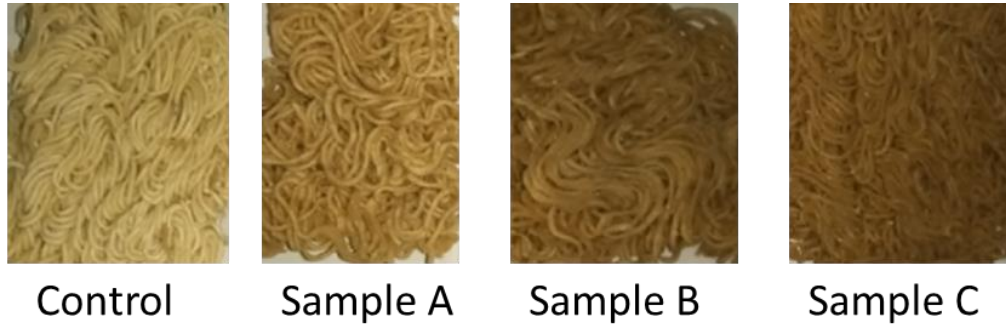


Figure 1. Image of instant noodles made from the different flour composites. Control was made with 100% wheat, Sample A with 80% wheat flour, 15% soybean flour, 5% carrot pomace flour, Sample B with 70% wheat flour, 20% soybean flour, 10% carrot pomace flour and Sample C with 65% wheat flour, 25% soybean flour, 15% carrot pomace flour.

Table 5. Color characteristics of instant fried noodles supplemented with soybean flour and carrot pomace flour.

Sample	L*	a*	b*
Control	55.910±1.434 ^a	-1.490±0.141 ^d	19.271±0.121 ^d
Sample A	44.633±1.589 ^b	5.121±0.458 ^c	27.912±1.333 ^a
Sample B	42.312±2.356 ^c	5.631±0.462 ^{ab}	26.812±0.213 ^b
Sample C	39.673±0.586 ^d	5.712±0.378 ^a	25.231±0.763 ^{bc}

Control= 100% wheat, Sample A=80% wheat flour, 15% soybean flour, 5% carrot pomace flour, Sample B = 70% wheat flour, 20% soybean flour, 10% carrot pomace flour, Sample C= 65% wheat flour, 25% soybean flour, 15% carrot pomace flour. Values are shown as mean ± standard deviation of triplicate analysis. Different superscript letters in a column indicate significant differences ($P \leq 0.05$).

Table 6. Textural properties of cooked instant fried noodles supplemented with soy flour and carrot pomace flour.

Sample	Tensile strength (N)	Breaking length (mm)
Control	0.280±0.01 ^a	36.512±1.013 ^a
Sample A	0.171±0.021 ^b	28.013±1.213 ^b
Sample B	0.153±0.215 ^{bc}	27.143±0.154 ^c
Sample C	0.121±1.314 ^c	24.165±0.314 ^d

Control= 100% wheat, Sample A=80% wheat flour, 15% soybean flour, 5% carrot pomace flour, Sample B = 70% wheat flour, 20% soybean flour, 10% carrot pomace flour, Sample C= 65% wheat flour, 25% soybean flour, 15% carrot pomace flour. Values shown are mean ± standard deviation of triplicate analysis. Different superscript letters in a column indicate significant differences ($P \leq 0.05$).

but was not significantly different from sample B. The yellowness (b^*) values also increase with the control being significantly different from the rest of the noodle samples.

Texture analysis

Textural characteristics of noodles are among the important consideration in determining their quality. The quality of the noodles is primarily defined by texture and

appearance (Ross, 2006). The textile profile of the noodles prepared from the composite flour is shown in Table 6. The tensile strength and breaking length decreased with substitution of the wheat flour. The control had the highest tensile strength and was significantly different from the noodles prepared from the composite flours. This decrease in tensile strength is ascribed to addition of non-gluten flours which weakens the gluten strength of the dough hence the overall structure of the noodles (Kovacs et al., 2004). Gluten make up 80% of the total protein in wheat flour. Gluten is

Table 7. Sensory analysis results of cooked instant fried noodles supplemented with soybean flour and carrot pomace flour.

Sample	Taste	Aroma	Color	Texture	Overall acceptability
Control	7.75 ± 0.753 ^b	7.083 ± 1.164 ^b	6.683±1.833 ^d	7.181± 1.601 ^b	6.416 ± 2.094 ^d
Sample A	8.01 ±1.206 ^a	7.510±1.314 ^a	7.751±0.965 ^a	7.272±1.793 ^a	7.916 ± 1.083 ^a
Sample B	6.583±1.564 ^c	7.083± 1.928 ^b	7.33±1.370 ^c	6.181±1.401 ^c	7.010±1.651 ^b
Sample C	5.833±1.589 ^d	6.583± 0.996 ^c	7.583± 1.24 ^b	5.363± 1.689 ^d	6.051±1.564 ^c

Control= 100% wheat, Sample A=80% wheat flour, 15% soybean flour, 5% carrot pomace flour, Sample B = 70% wheat flour, 20% soybean flour, 10% carrot pomace flour, Sample C= 65% wheat flour, 25% soybean flour, 15% carrot pomace flour. Values shown are mean± standard deviation of triplicate analysis. Different superscript letters in a column indicate significant difference (P≤ 0.05).

responsible for dough extensibility and strength, replacing wheat flour impairs the gluten matrix hence leading to the weakening of the noodle texture (Kovacs et al., 2004). Similar trend was reported by other studies who substituted wheat flour with Tilapia bone flour (Sirichokworakit, 2014) and unripe banana flour (Ritthiruangdej et al., 2011).

Sensory analysis

Sensory parameters of cooked noodles evaluated (taste, aroma, color, texture and overall acceptability) are shown in Table 7. Sample A with 80% wheat flour, 15% soybean flour and 5% carrot pomace was the most preferred by the panelist. Sample C scored the lowest score in taste (5.833), texture (5.363) and overall acceptability (6.051). The control scored the lowest in color. Sensory characteristics like smell, taste, appearance influence food acceptability more than the nutritive value. They play a significant role in the selection of a food item.

Conclusion

Instant fried noodles were prepared by substituting wheat flour with soybean (15, 20 and 25%) and carrot pomace flour (5, 10 and 15%) in an effort to improve their protein and dietary fiber content. Addition of soybean and carrot pomace flour in the noodles increased their protein, crude ash, crude fiber and total carotenoid content. Cooking time, cooking loss and water absorption of the prepared noodles increased with replacement of wheat flour. Lightness of the noodles decreased with replacement of wheat with soybean and carrot pomace flour. The control (100% wheat flour) had the highest tensile strength and was significantly different from the noodles prepared from the composite flours. Sensory analysis showed that noodles prepared from substitution of wheat with 15% soybean flour and 5% carrot pomace flour had the highest rating in taste, color, aroma, texture and overall acceptability. Soybean and carrot pomace flour can be used to formulate nutritious noodles with acceptable sensory properties.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Adegunwa M, Bakare H, Akinola OF (2012). Enrichment of Noodles with soy flour and carrot powder. *Nigerian Food Journal* 30(1):74-81.
- Adejuwon OH, Jideani AIO, Falade KO (2020). Quality and Public Health Concerns of Instant Noodles as Influenced by Raw Materials and Processing Technology. *Food Reviews International* 36(3):276-317.
- Ahmad M, Wani TA, Wani SM, Masoodi FA, Gani A (2016). Incorporation of carrot pomace powder in wheat flour: effect on flour, dough and cookie characteristics. *Journal of Food Science and Technology* 53:3715-3724.
- AOAC (2010). Official methods of analysis of AOAC International. Volume I, agricultural chemicals, contaminants, drugs / edited by William Horwitz. [Internet document] URL <https://repositorioinstitucional.ceu.es/handle/10637/3158>. Accessed 18/08/2020.
- Arora B, Kamal S, Sharma VP (2018). Nutritional and quality characteristics of instant noodles supplemented with oyster mushroom (*P. ostreatus*). *Journal of Food Processing and Preservation* 42(2):e13521.
- Asenstorfer RE, Wang Y, Mares DJ (2006). Chemical structure of flavonoid compounds in wheat (*Triticum aestivum* L.) flour that contribute to the yellow colour of Asian alkaline noodles. *Journal of Cereal Science* 43(1):108-119.
- Baljeet SY, Ritika BY, Reena K (2014). Effect of incorporation of carrot pomace powder and germinated chickpea flour on the quality characteristics of biscuits. *International Food Research Journal* 21(1):217-222.
- Collins JL, Pangloli P (1997). Chemical, physical and sensory attributes of noodles with added sweetpotato and soy flour. *Journal of Food Science* 62(3):622-625.
- Dewettinck K, Bockstaele F, Van Kühne B, Walle D, Van de Courtens TM, Gellynck X. (2008). Nutritional value of bread: Influence of processing, food interaction and consumer perception. *Journal of Cereal Science* 48(2):243-257.
- Faulks R, Southon S (2001). Carotenoids, Metabolism and Disease. *Handbook of Nutraceuticals and functional foods*. In: *Handbook of Nutraceuticals and functional foods* pp. 143-169.
- Fu B (2008). Asian noodles: History, classification, raw materials, and processing. *Food Research International* 41(9):888-902.
- Gulia N, Dhaka V, Khatkar BS (2014). Instant Noodles: Processing, Quality, and Nutritional Aspects. *Critical Reviews in Food Science and Nutrition* 54(10):1386-1399.
- Gull A, Prasad K, Kumar P (2015). Effect of millet flours and carrot pomace on cooking qualities, color and texture of developed pasta. *LWT - Food Science and Technology* 63(1):470-474.
- Gunathilake KDPP, Abeyrathne YMRK (2008). Incorporation of coconut flour into wheat flour noodles and evaluation of its Rheological,

- Nutritional and sensory characteristics. *Journal of Food Processing and Preservation* 32(1):133-142.
- Huh HJ, Shin WK, Kim Y (2019). Textural and cooking qualities of noodles made with soy flour and hydroxypropyl methylcellulose. *International Food Research Journal* 26(2):421-428.
- Hymavathi TV, Thejasri V, Roberts TPP (2019). Enhancing cooking, sensory and nutritional quality of finger millet noodles through incorporation of hydrocolloids. *International Journal of Chemical Studies* 7(2):877-881.
- Jalgaonkar K, Jha SK, Mahawar MK (2018). Influence of incorporating defatted soy flour, carrot powder, mango peel powder, and moringa leaves powder on quality characteristics of wheat semolina-pearl millet pasta. *Journal of Food Processing and Preservation* 42(4):e13575.
- Kang J, Lee J, Choi M, Jin Y, Chang D, Chang YH, Kim M, Jeong Y, Lee Y (2017). Physicochemical and Textural Properties of Noodles Prepared from Different Potato Varieties. *Preventive Nutrition Food Science* 22(3):246-250.
- Kim Y, Kim Y, Bae IY, Lee HG, Hou GG, Lee S (2013). Utilization of preharvest-dropped apple powder as an oil barrier for instant fried noodles. *LWT - Food Science and Technology* 53(1):88-93.
- Kovacs MIP, Fu BX, Woods SM, Khan K. (2004). Thermal stability of wheat gluten protein: Its effect on dough properties and noodle texture. *Journal of Cereal Science* 39(1):9-19.
- Kumoro AC, Johnny D, Alfilovita D (2016). Incorporation of microalgae and seaweed in instant fried wheat noodles manufacturing: nutrition and culinary properties study. *International Food Research Journal* 23(2):715-722.
- Lu Q, Guo S, Zhang S (2009). Effects of flour free lipids on textural and cooking qualities of Chinese noodles. *Food Research International* 42(2):226-230.
- Majzoobi M, Poor ZV, Jamaljan J, Farahnaky A (2016). Improvement of the quality of gluten-free sponge cake using different levels and particle sizes of carrot pomace powder. *International Journal of Food Science and Technology* 51(6):1369-1377.
- Medic J, Atkinson C, Hurburgh CR (2014). Current knowledge in soybean composition. *Journal of the American Oil Chemists' Society* 91:363-384.
- Omeire G, Umeji O, Obasi NE (2014). Acceptability of noodles produced from blends of wheat, acha and soybean composite flours. *Nigerian Food Journal* 32(1):31-37.
- Pakhare KN, Dagadkhair AC, Udachan IS, Andhale RA (2016). Studies on Preparation and Quality of Nutritious Noodles by Incorporation of Defatted Rice Bran and Soy Flour. *Journal of Food Processing and Technology* 7(10):629.
- Parveen H, Bajpai A, Bhatia S, Singh S (2017). Analysis of Biscuits Enriched With Fibre by Incorporating Carrot and Beetroot Pomace Powder. *The Indian Journal of Nutrition and Dietetics* 54(4):403-413.
- Prerana S, Anupama D (2020). Influence of carrot puree incorporation on quality characteristics of instant noodles. *Journal of Food Process Engineering* 43(3):e13270.
- Rajpurohit D (2018). Breeding Basmati Rice View project Book chapter View project A review: Food, chemical composition and utilization of carrot (*Daucus carota* L.) pomace. *International Journal of Chemical Sciences* 6(3):2921-2926.
- Rani S, Singh R, Kamble DB, Upadhyay A, Kaur BP (2019). Structural and quality evaluation of soy enriched functional noodles. *Food Bioscience* 32:100465.
- Rani S, Singh R, Kamble DB, Upadhyay A, Yadav S, Kaur BP (2020). Multigrain noodles: nutritional fitness and cost effectiveness for Indian Mid-Day Meal. *Food Security* 12:479-488.
- Rathod RP, Annapure US (2017). Physicochemical properties, protein and starch digestibility of lentil based noodle prepared by using extrusion processing. *LWT - Food Science and Technology* 80:121-130.
- Reddy KN, Duke SO (2015). Soybean Mineral Composition and Glyphosate Use. In: *Processing and Impact on Active Components in Food*. Elsevier Inc. pp. 369-376.
- Ritthiruangdej P, Parnbankled S, Donchedee S, Wongsagonsup R. (2011). Physical, chemical, textural and sensory properties of dried wheat noodles supplemented with unripe banana flour. *Kasetsart Journal - Natural Science* 45:500-509.
- Rodriguez-Amaya D, Kimura M (2004). HarvestPlus Handbook for Carotenoid Analysis. HarvestPlus Technical Monographs 59 p.
- Ross AS (2006). Instrumental measurement of physical properties of cooked Asian wheat flour noodles. *Cereal Chemistry* 83(1):42-51.
- Sahni P, Shere DM (2017). Physico-chemical and sensory characteristics of carrot pomace powder incorporated fibre rich cookies. *Asian Journal of Dairy and Food Research* 36(4):327-331.
- Semwal S, Chaudhary N, Karoulia S (2016). Addition of Carrot Pomace to Increase the Nutritional and Rheological Properties of Traditional Cake. *International Journal of Science and Research* 5:1412-1416.
- Sharma KD, Karki S, Thakur NS, Attri S (2012). Chemical composition, functional properties and processing of carrot-A review. *Journal of Food Science and Technology* 29:22-32.
- Siah S, Quail KJ (2018). Factors affecting Asian wheat noodle color and time-dependent discoloration - A review: *Cereal Chemistry* 95(2):189-205.
- Sirichokworrakit S (2014). Physical, textural and sensory properties of noodles supplemented with tilapia bone flour (*Tilapia nilotica*). *International Journal of Biological, Veterinary, Agricultural and Food Engineering* 8(7):689-691.
- Surbhi S, Verma RC, Deepak R, Jain HK, Yadav KK (2018). A review: Food, chemical composition and utilization of carrot (*Daucus carota* L.) pomace. *International Journal of Chemical Studies* 6(3):2921-2926.
- Takahashi T, Miura M, Ohisa N, Mori K, Kobayashi S (2005). Heat treatments of milled rice and properties of the flours. *Cereal Chemistry* 82(7):228-232.
- Udachan IS, Pakhare KN, Dagadkhair AC (2018). Enhancement of nutritional and functional characteristics of noodles by fortification with protein and fiber: A review. *Journal of Pharmacognosy and Phytochemistry* 7(1):351-357.
- Violalita F, Evawati, Syahrul S, Yanti HF, Fahmy K. (2020). Characteristics of Gluten-Free Wet Noodles Substituted with Soy Flour. In: *IOP Conference Series: Earth and Environmental Science*. P. 12047. Institute of Physics Publishing.
- Wandee Y, Uttapap D, Pancha-arnon S, Puttanlek C, Rungsardthong V, Wetprasit N (2014). Enrichment of rice noodles with fibre-rich fractions derived from cassava pulp and pomelo peel. *International Journal of Food Science and Technology* 49(11):2348-2355.
- Xu J, Bock JE, Stone D. (2020). Quality and textural analysis of noodles enriched with apple pomace. *Journal of Food Processing and Preservation* 44(8):e14579.
- Yadav S, Pathera AK, Islam RU, Malik AK, Sharma DP (2018). Effect of wheat bran and dried carrot pomace addition on quality characteristics of chicken sausage. *Asian-Australasian Journal of Animal Sciences* 31(5):729-737.
- Zhu F, Li J (2019). Physicochemical and sensory properties of fresh noodles fortified with ground linseed (*Linum usitatissimum*). *LWT* 101:847-853.

Full Length Research Paper

Increasing gallic acid contents in green tea extracts using acid hydrolysis

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Gallic acid (GA) is a functional ingredient abundant in Chinese pu-erh tea. The aim of this study was to increase the GA content in green tea extracts using acid hydrolysis. (-)-Epigallocatechin gallate treated with 1 M hydrochloric acid at 110°C for 1 h resulted in a GA yield of 45.6%. However, under these conditions, (-)-epigallocatechin was easily oxidized and rendered undetectable. On applying the same treatment to green tea extracts of Korea-cultivated *Chamnok*, a native species, and *Yabukita*, the GA contents increased from 0.17 to 4.87, 0.28 to 5.33 and 0.17 to 4.44 mM, respectively. In *Chamnok* extracts prepared following harvesting at three different time points, the GA contents increased from 0.17 to 4.48, 0.12 to 5.16 and 0.06 to 5.71 mM. Therefore, it is possible to produce green tea extracts with high GA concentrations using simple acid hydrolysis. This will greatly benefit the production of functional ingredients and will be useful in the beverage industry.

Key words: Domestic cultivar, EGCG, EGC, tannase.

INTRODUCTION

Green tea is consumed globally and has been reported to have beneficial effects on fat oxidation and energy

expenditure; it also favors weight loss (Kim et al., 2020). Green tea contains catechins, which represent a major

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component that can comprise up to approximately 30% of dry tea leaves (Chacko et al., 2010). The most abundant green tea catechins are (-)-epigallocatechin gallate (EGCG), which account for approximately 68–69% of all green tea catechins, followed by (-)-epigallocatechin (EGC, approximately 15–18% of all green tea catechins), (-)-epicatechin gallate (ECG, approximately 5–6% of all green tea catechins), and (-)-epicatechin (EC, approximately 2–5% of all green tea catechins) (Wang et al., 2019). The health benefits of green tea have been highly attributed to these polyphenolic compounds (Shang et al., 2020). *In vivo*, green tea catechins, especially EGCG, reduce the incidence of cancers, demonstrate antiviral activity, and reportedly ameliorate collagen-induced arthritis, oxidative stress-induced neurodegenerative diseases, and streptozotocin-induced diabetes (An et al., 2020; Hayakawa et al., 2020; Izzo et al., 2020; Mohan et al., 2020). In addition, EGCG tends to reduce body weight and fat.

During the fermentation process, gallate-type catechins such as EGCG or ECG are degraded, and gallic acid (GA, 3,4,5-trihydroxybenzoic acid) levels are markedly increased (Ge et al., 2019). GA is a characteristic component of fermented tea. It not only exerts antioxidant, anti-obesity, antibacterial, and anti-inflammatory effects, but also promotes the loss of body fat (Ge et al., 2019; Zhou et al., 2020). Furthermore, the Ministry of Food and Drug Safety in Korea has recognized the tea extract of pu-erh tea (containing GA as the standard component) as a functional health food ingredient. Green tea, a raw material of fermented tea, originally contained a small amount of GA, but also contained other types of catechins, such as EGCG, ECG, EGC, and EC (Kongpichichoke et al., 2016).

The three predominant green tea species cultivated in Korea are the native species, and the cultivars *Yabukita* and *Chamnok*. Native species accounted for the highest percentage of cultivation area, and *Yabukita* is typically processed mechanically, mostly employing the Jeju Island as its center. The production area of *Chamnok*, a domestic breed in Korea, is currently expanding. Both tannins and catechins are major contributors to the bitter taste of green tea, which are important indicators of tea quality, and their levels increased with an increased delay in harvesting. Conversely, the levels of free amino acids, theanine, and total nitrogen (all of which contributed to the pleasant taste of green tea) decreased with an increased delay in harvesting, resulting in a deterioration of quality (Jo et al., 2011). Isolated catechins appeared as a brown powder, which is extremely bitter and easily oxidizable; this hindered their application as a pharmaceutical agent (Dai and Mumper, 2010). Consequently, green tea leaves at the third harvest

(collected in August) are less desirable for beverage production (Cao et al., 2019). More recently, GA was found to contribute to the sweet aftertaste of a green tea infusion at the third harvest, following its biotransformation using tannase (Cao et al., 2019). Additionally, Kim et al. (2019, 2020) reported that EGC and GA complexes from EGCG or green tea infusion by tannase biotransformation exert an anti-obesity effect in brown adipose tissues.

Therefore, this study attempts to improve the functionality and aftertaste of EGCG in pu-erh tea using a simple acid-based chemical reaction by replacing biotransformation with tannase or long-time fermentation. In this study the aim was to produce a green tea extract with an increased GA content through the acid hydrolysis of EGCG or a green tea infusion.

MATERIALS AND METHODS

Materials

The green tea leaves were supplied by Jeonnam Agricultural Research & Extension Services Centre (Bosung, Korea) and by the Agricultural Research Centre for Climate Change (Jeju, Korea). For use as standards, EC, ECG, EGC, EGCG, and catechin (C) were purchased from Aktin Chemicals Inc. (Cheng-du, China), and GA was purchased from Tokyo Chemical Industry Co. (Chuo-ku, Tokyo, Japan). All standards had a purity of >95%.

Preparation of EGCG solution and green tea extracts

The EGCG solution (at a concentration of 0.2 M) was prepared by shaking at 25°C for 2 h in the dark. Green tea powder (5 g) was dissolved in 250 ml of 60% (v/v) ethanol, followed by homogenization and incubation at 25°C for 1 h in the dark (Kim et al., 2019). After filtration through a No. 2 Whatman filter paper (GE Healthcare, Buckinghamshire, UK), the remaining insoluble residue was added to 250 ml of 100% (v/v) ethanol, followed by incubation under the same conditions. The total filtrate was concentrated to a volume of 30 ml using 60% (v/v) ethanol. This green tea extract was used for hydrolysis experiments.

Acid hydrolysis of green tea extracts and identification of catechins by high-performance liquid chromatography (HPLC)

EGCG was acid-hydrolyzed using different catalysts, treatment times, and temperature conditions. Both the EGCG solution and green tea extract were subjected to hydrolysis using HCl (0.025–1 M) for 5, 15, 30, 45, or 60 min at 110°C, as previously described at various temperatures (Zhang et al., 2014). After cooling on ice, EGCG solution-acid mixtures were neutralized using 1 M sodium hydroxide, prior to lyophilization and resuspension, for HPLC analysis. Dried samples were dissolved in 100 µl of solvent A (20 mM phosphoric acid in water); they were diluted, filtered through a 0.20-µm, filtered (DISMIC-13HP, Advantec, Tokyo, Japan), and injected onto a HPLC column (Shimadzu, Tokyo, Japan) equipped

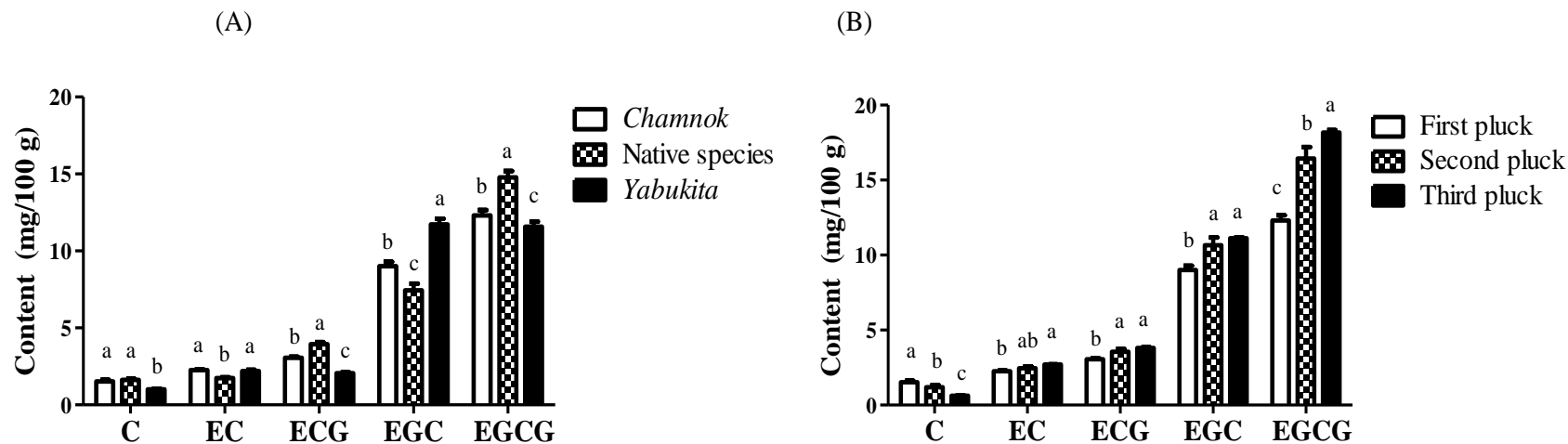


Figure 1. Content of catechins in *Chamnok* green tea extracts prepared from various cultivars (A) and with different plucking times (B). Data means with different letters in the same column are significantly different ($p < 0.05$), as determined by Duncan's multiple range test. The concentrations of GA, EGC, and EGCG were analyzed by HPLC. C, (-)-catechin; EC, (-)-epicatechin; EGC, (-)-epigallocatechin; ECG, (-)-epicatechin gallate; EGCG, (-)-epigallocatechin gallate.

with a Shodex Silica C18P 4D column (5μ , 4.6×150 mm, Shodex, Tokyo, Japan) pumps A and B (LC-20AD, Shimadzu, Kyoto, Japan), and a UV-VIS detector (SPD-20A, Shimadzu, Kyoto, Japan). Catechins and GA were eluted using a mobile phase composed of a mixture of 20 mM phosphoric acid in water (phase A) and acetonitrile (phase B), at a flow rate of 1 ml/min. The linear gradient dynamics were as follows: phase B increased from 0 to 15% (v/v) over 5 min, maintained at 15% (v/v) for 10 min, increased from 15 to 40% (v/v) over 15 min of a mixture of 20 mM phosphoric acid in water (phase A) and a (v/v) over 25–30 min, and then maintained at 0% (v/v) for an additional 5 min. The UV-VIS detector wavelength was set to 280 nm, and the identification of individual catechins and GA was based on a comparison of peak retention times of the samples with those of the reference standards. The concentrations of green tea constituents were estimated from the area under the curve (Baik et al., 2015).

Statistical analysis

All experiments were performed in triplicate and the data were analyzed for statistical significance using a one-way analysis of variance (ANOVA), followed by Duncan's multiple range test [SPSS version 18.0 (IBM Co., Armonk, NY, USA)]. The significance level was set at $p < 0.05$. Mean values obtained from each group were compared using ANOVA.

RESULTS AND DISCUSSION

Catechin and GA contents in green tea extracts

Polyphenols, especially catechins and phenolic

acids are considered the major mediators of the health effects presented by green tea. The EGCG, EGC, or GA contents varied depending on the Korean domestic cultivar. As shown in Figure 1, phenolic compounds constituted approximately 30–40% of the dry weight of green tea leaves. Consistent with findings reported in a previous study (Wang et al., 2019), assessment of the catechin content in green tea extracts from various cultivars revealed that EGCG was the most abundant, followed by EGC, EC, and C. Furthermore, although EGCG and EGC were the most abundant among the native species and in the cultivar *Yabukita*, respectively, EGCG, GA, and C levels did not differ significantly among the

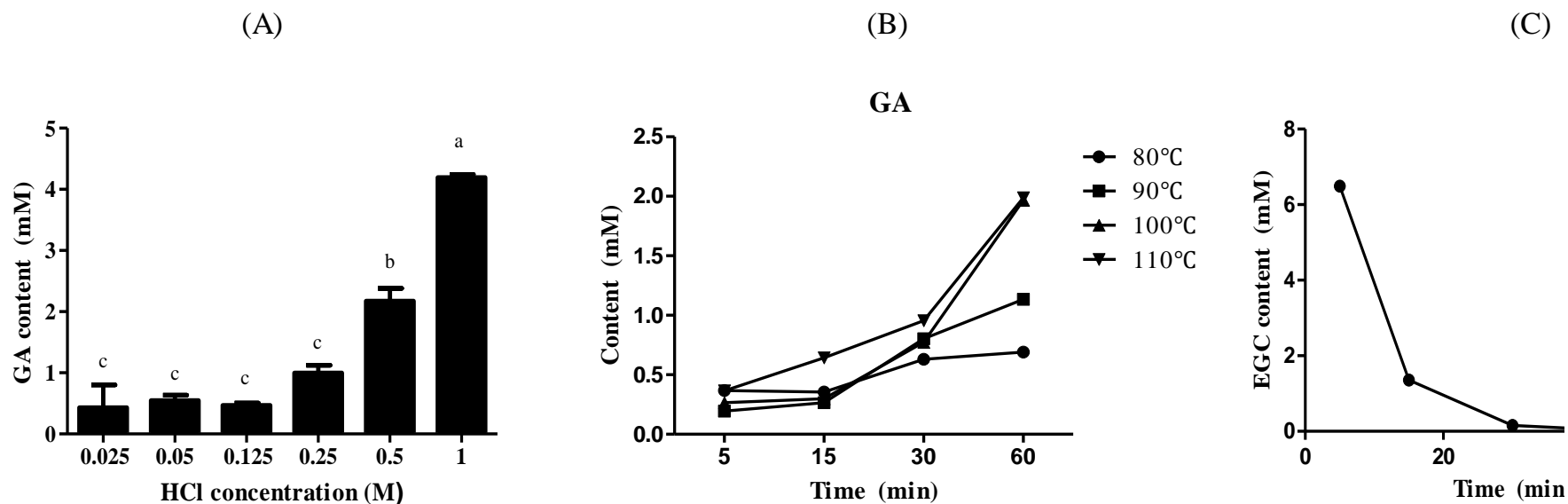


Figure 2. GA contents produced after treatment with different HCl concentrations (A), GA contents produced after treatment with different temperatures (B), and EGC contents after acid hydrolysis at 110°C for 1 h (C). Data means with different letters in the same column are significantly different ($p < 0.05$), as determined by Duncan's multiple range test. The concentrations of GA, EGC, and EGCG were analyzed by HPLC. GA, gallic acid; EGC, (-)-epigallocatechin; EGCG, (-)-epigallocatechin gallate.

three cultivars evaluated. The levels of catechins, phenolic acids, and caffeine in commercial teas varied with the species, season, horticultural conditions, and particularly, the degree of fermentation (Yang et al., 2018). In the *Chamnok* green tea extract, we observed that the content of catechins (except C) increased when the harvesting (plucking) period was delayed (Figure 1B). Accordingly, for *Chamnok*, the EGCG content gradually increased based on the plucking time (from April to August).

Investigation of the acid hydrolysis conditions for EGCG

Depending on the HCl concentration, EGCG was gradually hydrolyzed up to a concentration of 1 M HCl (Figure 2A). Consequently, the GA content was highest at 110°C for 1 h, revealing a GA yield of 45.6% of the initially added EGCG (Figure 2B). Additionally, EGCG was not hydrolyzed following exposure to mild acids such as acetic, lactic, or citric acid, but it was degraded into GA and EGC

in an HCl concentration-dependent manner under heating conditions (Figure 2A). Lee et al. (2012) have demonstrated that radical-mediated oxidation of EGCG can result in a low EGCG yield. Furthermore, acid hydrolysis did not increase the EGC content in the EGCG solution over time, and EGC was undetectable in the green tea infusion after acid treatment, indicating that it is unstable under acidic heating conditions (Figure 2C). Additionally, GA was not produced at a yield exceeding 50%, possibly due to a radical reaction.

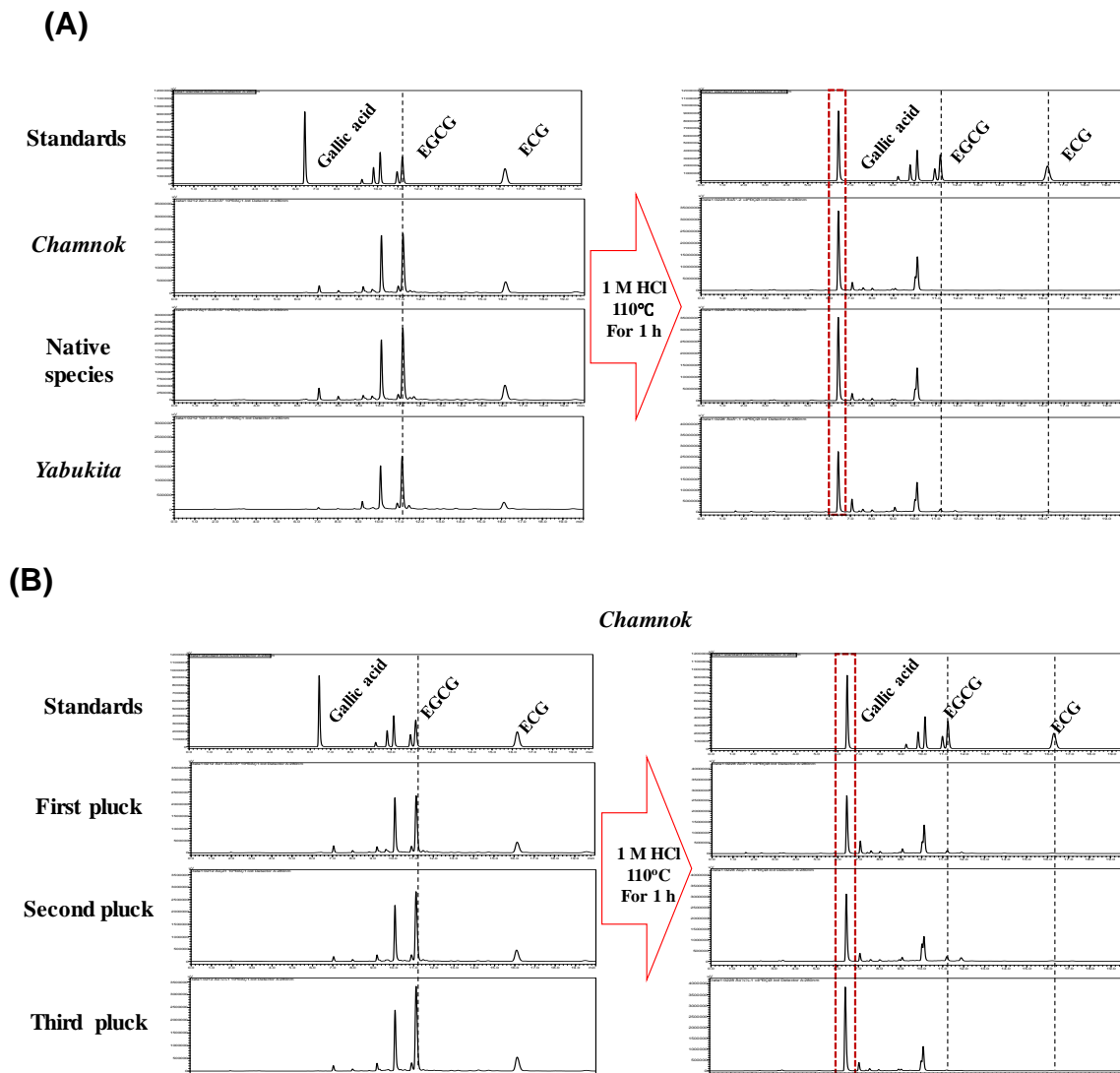


Figure 3. Contents of GA, EGC, and EGCG after acid hydrolysis of green tea extracts prepared from various cultivars (A) and with different plucking times at 110°C (B). GA, gallic acid; EGC, (-)-epigallocatechin; EGCG, (-)-epigallocatechin gallate.

Similar results were obtained in the experiment investigating the impact of different cultivars (Figure 3A) and harvesting (plucking) time points on the content of EGCG in the green tea extract (Figure 3B). However, the GA yield was high owing to the presence of multiple gallated catechins (ECG, GCG, and EGCG). Indeed, in the green tea extract, the contents of EGCG and ECG were significantly reduced, whereas the GA content was enhanced following acid hydrolysis at 110°C. The GA content increased from 0.17 to 4.87 mM (28.64-fold), 0.28 to 5.33 mM (19.04-fold), and 0.17 to 4.44 mM

(26.12-fold) in the green tea extracts prepared from *Chamnok*, a native species, and *Yabukita*, respectively (Table 1).

The content of catechins increased with an increasing delay in harvesting (plucking) time, and subsequent acid hydrolysis proportionately increased the GA levels in relevant green tea extracts. In case of the Korea-cultivated *Chamnok*, the GA content increased from 0.17 to 4.48 mM (22.35-fold), 0.12 to 5.16 mM (43-fold), and 0.06 to 5.71 mM (95.17-fold) in green tea extracts prepared from the first (April), second (June), and third

Table 1. Catechin content changes after acid hydrolysis, in the green tea infusion depending on the cultivar or plucking times.

Cultivar	Non-treatment			Acid hydrolysis			
	GA (mM)	EGCG (mM)	ECG (mM)	GA (mM)	EGCG (mM)	ECG (mM)	
<i>Chamnok</i>	0.17±0.05 ^{NS}	3.49±0.00 ^{NS}	1.70±0.03 ^b	4.87±0.10 ^{NS}	0.01±0.00 ^{NS}	0.03±0.00 ^{NS}	
Native species	0.28±0.12 ^{NS}	3.37±0.25 ^{NS}	2.13±0.13 ^a	5.33±0.59 ^{NS}	0.00±0.00 ^{NS}	0.03±0.00 ^{NS}	
<i>Yabukita</i>	0.17±0.09 ^{NS}	3.43±0.09 ^{NS}	1.16±0.03 ^c	4.44±0.02 ^{NS}	0.00±0.00 ^{NS}	0.05±0.00 ^{NS}	
Cultivar	Plucking times	GA (mM)	EGCG (mM)	ECG (mM)	GA (mM)	EGCG (mM)	ECG (mM)
	First	0.17±0.03 ^a	3.43±0.07 ^c	1.10±0.02 ^{NS}	4.48±0.33 ^b	0.00±0.00 ^{NS}	0.03±0.02 ^{NS}
<i>Chamnok</i>	Second	0.12±0.0 ^b	4.57±0.00 ^b	1.24±0.19 ^{NS}	5.16±0.16 ^{ab}	0.01±0.00 ^{NS}	0.04±0.03 ^{NS}
	Third	0.06±0.00 ^c	5.20±0.08 ^a	1.25±0.22 ^{NS}	5.71±0.55 ^a	0.01±0.00 ^{NS}	0.03±0.02 ^{NS}

Data presented are means ± standard deviation from triplicate measurement and means with the different letters in the same column are significantly different ($p < 0.05$) as determined by Duncan's multiple range test. GA, gallic acid, EGC, (-)-epigallocatechin; EGCG, (-)-epigallocatechin gallate; NS, not significantly different.

(August) harvests (plucks), respectively (Table 1).

Conclusion

In this study an attempt was made to improve the GA contents in EGCG solutions or green tea infusions using a simple acid-based chemical reaction, and expanded the same process using domestically cultivated cultivars or different harvesting times of green tea. There was GA yield of 45.6% after treatment with 1 M HCl at 110°C for 1 h. Following acid hydrolysis, GA levels improved by 19-, 26-, and 28-fold in green tea infusions of the native species, *Yabukita*, and *Chamnok*, respectively, compared with those observed before treatment. Compared with tannase treatment, acid hydrolysis did not lead to the production of EGC or EC from EGCG or ECG, owing to their instability under conditions of strong acid hydrolysis. Although, EGC or EC was not obtained, acid hydrolysis is a simple and cost-effective process to increase the GA content in green tea infusions. Based on the present results, the high concentration of GA in green tea infusions can be replaced with several processes in pu-erh tea that require more than six months to over 10 years to ferment. Thus, the production of green tea solutions with high GA contents will have potential applications in manufacturing functional ingredients and beverages at an industrial level.

ACKNOWLEDGEMENT

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CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

ABBREVIATIONS

EC, (-)-epicatechin; **ECG**, (-)-epicatechin gallate; **EGC**, (-)-epigallocatechin; **EGCG**, (-)-epigallocatechin gallate; **GA**, gallic acid.

REFERENCES

- An L, Zhang W, Ma G, Wang K, Ji Y, Ren H, Wang Y (2020). Neuroprotective effects of *Camellia nitidissima* Chi leaf extract in hydrogen peroxide-treated human neuroblastoma cells and its molecule mechanism. *Food Science and Nutrition* 8(9):4782-4793.
- Baik JH, Shin KS, Park Y, Yu KW, Suh HJ, Choi HS (2015). Biotransformation of catechin and extraction of active polysaccharide from green tea leaves via simultaneous treatment with tannase and pectinase. *Journal of the Science of Food and Agriculture* 95:2337-2344.
- Cao QQ, Zou CH, Zhang YH, Du QZ, Yin QZ, Yin JF, Shi J, Xue S, Xu YQ (2019). Improving the taste of autumn green tea with tannase. *Food Chemistry* 277:432-437.
- Chacko SM, Thambi PT, Kuttan R, Nishigaki I (2010) Beneficial effects of green tea: a literature review. *Chinese Medicine* 5:13.
- Dai J, Mumper RJ (2010) Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules* 15:7313-7352.
- Ge Y, Bian X, Sun B, Zhao M, Ma Y, Tang Y, Li N, Wu JL (2019). Dynamic profiling of phenolic acids during pu-erh tea fermentation using derivatization liquid chromatography-mass spectrometry approach. *Journal of Agricultural and Food Chemistry* 67:4568-4577.
- Hayakawa S, Ohishi T, Miyoshi N, Oishi Y, Nakamura Y, Isemura M (2020). Anti-cancer effects of green tea epigallocatechin-3-gallate and coffee chlorogenic acid. *Molecules* 25: 4553.
- Izzo S, Naponelli V, Bettuzzi S (2020). Flavonoids as epigenetic modulators for prostate cancer prevention. *Nutrients* 12:1010.
- Jo JS, Kim JC, Cho KH, Kim RM, Han JY (2011). Chemical constituent variabilities of the green tea leaves by harvest periods. *Journal of the Korean Wood Science and Technology* 39:370-380.

- Kim HS, Jeon DY, Javid HMA, Sahar NE, Lee HN, Hong SJ, Huh JY, Kim YM (2020). Biotransformation of green tea infusion with tannase and its improvement on adipocyte metabolism. *Enzyme Microbial Technology* 135:109496.
- Kim HS, Moon JH, Kim YM, Huh JY (2019). Epigallocatechin exerts anti-obesity effect in brown adipose tissue. *Chemistry and Biodiversity* 16:e1900347.
- Kongpichichoke T, Chiu MT, Huang TC, Hsu JL (2016). Gallic acid content in Taiwanese teas at different degrees of fermentation and its antioxidant activity by inhibiting PKC δ activation: *In vitro* and in silico studies. *Molecules* 21:1346.
- Lee HJ, Cho JY, Moon JH (2012). Chemical conversions of salvianolic acid B by decoction in aqueous solution. *Fitoterapia* 83:1196-1204.
- Mohan T, Narasimhan KKS, Ravi DB, Velusamy P, Chandrasekar N, Chakrapani LN, Srinivasan A, Karthikeyan P, Kannan P, Tamilarasan B, Johnson T, Kalaiselvan P, Periandavan K (2020). Role of Nrf1 dysfunction in the pathogenesis of diabetic nephropathy: Therapeutic prospect of epigallocatechin-3-gallate. *Free Radical Biology and Medicine* 160:227-238.
- Shang A, Liu HY, Luo M, Xia Y, Yang X, Li HY, Wu DT, Sun Q, Geng F, Li HB, Gan RY (2020). Sweet tea (*Lithocarpus polystachyus* rehd.) as a new natural source of bioactive dihydrochalcones with multiple health benefits. *Critical Reviews in Food Science and Nutrition*, pp. 1-18.
- Wang Y, Kan Z, Wang D, Zhang L, McGinley JN, Thompson HJ (2019). Differences in chemical composition among commercially important cultivar of genus *Camellia*. *Journal of Agricultural and Food Chemistry* 67:5457-5464. <https://doi.org/10.1021/acs.jafc.8b06164>
- Yang C, Hu Z, Lu M, Li P, Tan J, Chen M, Lv H, Zhu Y, Zhang Y, Guo L, Peng Q, Dai W, Lin Z (2018). Application of metabolomics profiling in the analysis of metabolites and taste quality in different subtype of white tea. *Food Research International* 106:909-919. <https://doi.org/10.1016/j.foodres.2018.01.069>
- Zhang L, Wang Y, Xu M (2014). Acid hydrolysis of crude tannins from infructescence of *Platycarya strobilacea* Sieb. et Zucc to produce ellagic acid. *Natural Product Research* 28:1637-1640.
- Zhou B, Ma C, Ren X, Xia T, Zheng C, Liu X (2020). Correlation analysis between filamentous fungi and chemical compositions in a pu-erh type tea after a long-term storage. *Food Science and Nutrition* 8:2501-2511.

Full Length Research Paper

Assessment of phytochemical and mineral composition of unripe and ripe plantain (*Musa paradisiaca*) peels

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Plantain (*Musa paradisiaca*) fruit constitutes a staple food widely consumed predominantly in Africa. The peel, a major by-product, of plantain fruit is largely viewed to be of little or no significance and consequently discarded, thereby constituting a threat to the environment. It is on account of the foregoing that this study was designed to investigate the phytochemical and mineral components of both the unripe and ripe plantain peels, and possibly suggest ways for its proper utilization. This study was conducted using standard phytochemical assay procedures and the atomic absorption spectrophotometric methods. The result of the phytochemical screening showed the presence of alkaloids (3.53 ± 0.64 and 3.4 ± 0.38 g/100 g), flavonoids (0.16 ± 0.05 and 0.13 ± 0.02 g/100 g), tannins (2.18 ± 0.63 and 3.22 ± 0.82 g/100 g) and terpenoids (1.88 ± 0.24 and 1.83 ± 0.19 g/100 g) in unripe and ripe plantain peels, respectively. More so, for both the unripe and ripe plantain peels, considerable levels of Ca (176.30 ± 8.77 and 176.42 ± 8.94 mg/100 g), Na (47.37 ± 5.82 and 47.34 ± 5.72 mg/100 g), K (787.70 ± 6.20 and 787.73 ± 6.29 mg/100 g), Mg (81.60 ± 0.12 and 81.31 ± 0.31 mg/100 g), and Fe (40.95 ± 15.61 and 26.25 ± 14.80 mg/100 g) were detected in the peel samples investigated, respectively, with the unripe plantain however possessing a significantly higher level of Fe. The amount of Pb (0.4 ± 0.02 mg/100 g) and (0.023 ± 0.01 mg/100 g) for unripe and ripe, respectively, were significantly low ($P < 0.05$) to engender any deleterious consequences. This study therefore demonstrates that, the often undervalued plantain peels contain a substantial amount of mineral elements, phytochemicals and an infinitesimal level of toxicants. The peels, as a result, could be further processed and utilized as nutraceuticals in food and animal feeds.

Key words: Mineral elements, phytochemicals, plantain peels.

INTRODUCTION

In developing countries, the management of waste is a major difficulty faced by agro-based industries. In Nigeria, for example, plant biomasses are often incinerated in the open air regardless of the environmental implications (Babayemi et al., 2010). According to Tchobanoglous et

al. (1993), the public are exposed to serious health hazards resulting from poor and inefficient solid waste disposal. These include but not limited to environmental pollution and increase in insect vectors of disease. Tchobanoglous et al. (1993) expressed further that, the

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failure to remediate and utilize these materials ultimately leads to superfluous build-up of waste and natural resource depletion.

M. paradisiaca is an important staple food crop in Central and West Africa. The estimates of the Food and Agriculture Organization indicate that Nigeria produces about 2.11 million metric tons annually, and this contributes considerably to the diet of the local population (FAO, 2005). Plantain belongs to the genus *Musa* and can be consumed in both the unripe and ripe stages.

Wolfe et al. (2003) opined that the main by-product derived during the processing of fruits is the peels; and, in myriad instances these peels have proven a good source of dietary fibres, carotenoids, polyphenols and other bioactive compounds which are beneficial to human health. The work of Wolfe et al. (2003) preceded that of Ajasin et al. (2006) where it was chronicled that, plantain peels are of substantial nutritional value because it possess about 16% crude fibre, 12% crude protein and 1300 Kcal/kg on the basis of dry matter. Ajasin et al. (2006) further observed that whilst the quantity of starch decreased from 50 to 35%, the amount of total sugar increased from 3.0 to 31.6% in the peel during ripening. Even so, plantain peel is considered a rich source of hemicellulose (13%) and cellulose (10%) than its pulp which contains 1.3 and 1.4% for hemicellulose and cellulose, respectively (Agama-Acevedo et al., 2015).

Plants generally contain some biologically active naturally occurring chemicals also referred to as "secondary metabolites" (Zenk, 1996). It is yet well documented in the literature that, some of these bioactive substances in plants essentially serve as medicinal herbs (Soetan, 2008). Consequent upon this, several research works have been conducted to assess the phytochemical and mineral content of various parts of diverse plants to, among other things, unearth viable therapeutic alternatives to synthetic drugs. These phytochemicals include: alkaloids, flavonoids, saponins, tannins etc. Soetan (2008) advanced that some of these phytochemicals are applied as pharmacologically active agents and in nutrition.

Because of harsh weather conditions, appreciable amount of plantains are lost during post-harvest period due to accelerated ripening by warm weather. This therefore makes it imperative to bring out a way of making use of the ripe plantain peel. Additionally, it is equally on record that apart from the use of unripe plantain peel in soap making and possibly animal feeds, the usefulness of the ripe plantain peel, by contrast, has not been effectively maximized (Onyegbado et al., 2002). The foregoing observation is indeed unfortunate in this era of nutraceuticals and functional foods.

Also, besides the obvious use of these peels, most literature research studies lay little or no stress on the chemical composition of these peels, how they can be harnessed and bio-converted into useful materials. For this reason, this work sought to find out the phytochemical

and mineral composition of unripe and ripe plantain peels separately and comparatively in an attempt to promote its bioconversion into value added products.

MATERIALS AND METHODS

Chemicals

Concentrated sulphuric acid, Acetone, Sodium hydroxide, Boric acid, Ethanol, Concentrated HCL, Folin-Ciocalteau's reagent were products of Sigma Aldrich, St. Louis, MO, USA. All other chemicals, including the distilled water utilized, were of analytical grade.

Plant collection and extract preparation

The plantain samples (unripe and ripe) utilized for this research were purchased from Sabon Gari market, Kano, Nigeria. And the botanical identification and verification were carried out at the Department of Botany, Bayero University, Kano.

The unripe and ripe peels of plantain were subsequently removed. The peels were then collected, grouped and properly washed under running tap water to rid them of any contaminants. They were further rinsed with distilled water. Thereafter, these peels were sliced into small pieces (0.5 to 1.0 mm) and immediately air dried at 25°C for 240 h. The dried peels were ground into powder and passed through 0.1 mm mesh size sieve. The free-flowing powder samples were then packed into screwed bottles and labeled appropriately.

5 g of each powdered samples was consequently soaked in 500 ml solvent, distilled water and ethanol, as the case may be. The mixture was left to stand for about 48 h during which it was shaken intermittently. It was then filtered and the extract evaporated under reduced pressure at 40°C, whilst final solvent elimination was actualized with the aid of water bath.

Phytochemical screening

Phytochemical evaluation of peel samples was conducted on the aqueous, ethanolic and dried powder specimens according to standard procedures (Earl and Warren, 1961; Felgis, 1975; Sofowora, 1979 and 1993).

Mineral composition determination

Mineral elements were determined after 1 g of the ashed peel samples were subjected to wet oxidation using perchloric acid and nitric acid in accordance with the method of Osborn and Voogt (1978). The estimates of sodium and potassium in the digested samples were obtained using flame photometer (Model 52-A, Perkin Elmer, Waltham, MA, USA) in line with the procedure of Bonire et al. (1990).

The atomic absorption spectrophotometer (Analyst 200, Perkin Elmer, Waltham, MA, USA) was used to analyze the concentration of calcium, copper, magnesium, lead, zinc and iron according to the method of Bamhard (1985). The absorption of these mineral elements was compared with their respective standard absorption.

Statistical analysis

All analysis was conducted in triplicates. The results obtained therein were used for the statistical analysis using the statistical

Table 1. Qualitative screening of phytochemicals in aqueous and ethanolic extract of unripe and ripe plantain peels.

Phytochemicals	Aqueous extract		Ethanolic extract	
	Unripe peels	Ripe peels	Unripe peels	Ripe peels
Alkaloids	-	-	+	+
Flavonoids	-	-	+	+
Tannins	+	+	+	+
Saponins	+	+	-	-
Antraquinones	-	-	-	-
Cardiac glycosides	-	-	-	-
Terpenoids	-	-	+	+

+: Present, -: Absent.

Table 2. Quantitative estimate of phytochemicals present in ethanolic extract of unripe and ripe plantain peel (g / 100 g).

Phytochemicals	Ethanolic extract	
	Unripe peels	Ripe peels
Alkaloids	3.53 ± 0.64 ^a	3.47 ± 0.38 ^a
Flavonoids	0.16 ± 0.05 ^a	0.13 ± 0.02 ^a
Tannins	2.18 ± 0.63 ^a	3.22 ± 0.82 ^b
Terpenoids	1.88 ± 0.24 ^a	1.83 ± 0.19 ^a

The results represent the mean of three determinations ± standard deviation. Data on the same row with different superscripts are significantly different ($P < 0.05$).

package for social sciences (SPSS), version 20 (SPSS Inc., Chicago, IL, USA). Descriptive statistics and student t-test were used to analyze results.

RESULTS AND DISCUSSION

The objective of this study was to assess the phytochemical profile and mineral composition of unripe and ripe plantain peels separately and comparatively. The qualitative phytochemical evaluation was conducted in solvent extracts of unripe and ripe *M. paradisiaca* peel using distilled water and ethanol (Table 1), whilst only ethanolic dried powder specimen was subjected to quantitative screening (Table 2). The ashed peel samples were equally investigated for mineral element composition (Table 3).

According to Ighodaro et al. (2009), the medicinal properties of plants are largely informed by the bioactive compounds inherent in them. In this study, it was observed that phytochemicals like saponins and tannins were present in both the aqueous and ethanolic extracts of unripe and ripe plantain peels (Table 1). Surprisingly however, alkaloids, flavonoids and terpenoids were exclusively detected in only the ethanolic extract of the samples investigated (Table 1). It is notwithstanding plausible to rationalize that, the exclusive discovery of alkaloids, flavonoids and terpenoids in only the ethanolic

peel extract could be attributed to the different polarities of the solvents used for extraction.

Beyond that, the composition of these phytochemicals does not however seem to have been quantitatively influenced by ripening which is often marked by chemical changes. Besides the concentration of tannins that was found to be significantly higher ($P < 0.05$) in ripe plantain peels, no significant difference ($P > 0.05$) was observed in the level of alkaloids, flavonoids and saponins in the unripe and ripe plantain peels investigated (Table 2). Middleton and Kandaswami (1992) had advanced that phytochemicals induce myriad biological and pharmacological activities. Soforowa (1993) further corroborated Middleton and Kandaswami's hypothesis by stating that alkaloids, flavonoid and tannins possess enormous medicinal properties. According to Koleva et al. (2002), flavonoids inhibit free radical generation by interfering with biochemical pathways leading to ROS production. And hence are considered potent antioxidants.

Ojo et al. (2006) reported that, saponins possess antimicrobial properties and could function as precursors of several steroidal substances with diverse physiological roles. The discovery of saponins in the peels extract of plantain also renders it ideal for utilization in the preparation of medicinal soap given that saponin could function as a forming agent. This essentially could assist

Table 3. Mineral composition of unripe and ripe plantain peels (mg / 100 g).

Mineral element	Samples	
	Unripe peels	Ripe peels
Sodium	47.37 ± 5.82 ^a	47.34 ± 5.72 ^a
Calcium	176.30 ± 8.77 ^a	176.42 ± 8.94 ^a
Magnesium	81.60 ± 0.12 ^a	81.31 ± 0.31 ^a
Zinc	2.51 ± 0.48 ^a	3.01 ± 0.57 ^b
Iron	40.95 ± 15.61 ^a	26.25 ± 14.80 ^b
Potassium	787.70 ± 6.20 ^a	787.73 ± 6.29 ^a
Copper	1.19 ± 0.01 ^a	1.01 ± 0.01 ^a
Lead	0.4 ± 0.02 ^a	0.23 ± 0.01 ^b

The results represent the mean of three determinations ± standard deviation. Data on the same row with different superscripts are significantly different ($P < 0.05$).

in reducing the utilization of synthetic forming agents in medicinal soaps (Wijetunge and Perera, 2016). In a paper by Asquith and Butler (1986), it was posited that tannins confer anti-hemorrhagic and anti-diarrhoeic properties. These observations, taken together, presuppose that the unripe and ripe plantain peels contain invaluable therapeutic potential yet to be optimized.

Refreshingly, plantain peels seem to be a promising source of nutrients for animal feeds production (Babatunde, 1992). Calles et al. (2000) reported that in the diet of growing pig, meals made from ripe plantain peel replaced about 31% of maize without any adverse condition on growth performance. This could possibly be linked to the disease preventive nay protective effect of the putative non-nutritive chemicals present in this peel.

It has been revealed by this research that plantain peels are high in potassium, sodium and calcium, and hence could be considered a vital source of these elements for both plants and animals nutrition. In quantitative terms, a relatively high level of calcium, magnesium, potassium and sodium were discovered in both peels (Table 3). There was nonetheless any significant difference ($P > 0.05$) in the content of these mineral elements. In one twist however, unripe plantain peels demonstrated a significantly higher ($P < 0.05$) level of iron and lead (Table 3). While it is true that these findings are consistent with the observations of Ighodaro (2012), it is even yet promising that the levels obtained were in conformity with WHO recommendations.

O'Connell (2011) posited that calcium is an essential part of intracellular processes that take place inside insulin responsive tissues like the adipose tissue and skeletal muscle. Changes in calcium flux can result in deleterious consequences on insulin secretion, a calcium dependent process. Therefore, the considerable level of calcium in the peels of unripe and ripe plantain in this study hints the significance of these peels to diabetics.

Chen et al. (2010) linked the incidence of hypertension to the intake of diet with higher Na to K ratio.

Interestingly, the relatively higher amount of K to Na observed in this work could serve a comparative advantage on grounds of Chen's observation. Alternative revelation from this investigation is that, K is the predominant mineral element in plantain peels.

Combustion is the primary process for directly utilizing biomass energy with consequent production of ash as by-product. The ash of plantain peels possesses an enormous quality that qualifies it for wide range of applications in agriculture (Onyegbado et al., 2002). According to Israel and Akpan (2016), ashes from these peels could be utilized in the production of NPK fertilizer. The plantain peel nay biomass is combusted in hot fire to produce a white ash and Potassium oxide is extracted from the ash thereof. This procedure is more economical than obtaining KOH from the market. The potash obtained from plantain peels is utilized for local production of soap and is equally a potential chemical for biodiesel production, hence making plantain peels a potential renewable energy source (Enontimonria et al., 2016; Betiku and Sheriff, 2014).

Pyruvate kinase utilizes magnesium as a cofactor. Beyond this, magnesium also regulates glucose transport across cell membrane (O'Connell, 2011). With the discovery of no significant loss of magnesium in the samples studied, the usefulness of these peel samples is even yet established. The role of zinc in the control of insulin production by pancreatic tissues and the use of glucose by fat and muscle cells was comprehensively elucidated by Eleazu et al. (2013). Observation from this study reveals a significantly higher level of zinc in the ripe plantain peel. This revelation is, however, confounding as Okorie et al. (2015) reported diametrically opposite results.

Copper is a trace element found in living organisms and crucial not just in redox chemistry, but also in growth and development. Today, this element is being explored as a therapy for several conditions, including neurodegenerative disorders like Alzheimer and Parkinson disease (Tisato et al., 2010).

Exposure to heavy metals like lead, mercury, cadmium and arsenic yet remains a huge threat to human health (Jarup, 2003). Lead is obviously toxic to kidneys, heart, intestines, bone, nervous and reproductive systems. It specifically impairs nervous system development and thus toxic to children, resulting in permanent behavior and learning disorders (Jarup, 2003). Most importantly, the detection of considerable level of iron in unripe plantain peel tends to be a significant discovery in this study. Chen et al. (2010) contended that iron is an important component of hemoglobin and it is critical in the production of energy and normal function of the immune system.

Conclusion

This study has revealed that unripe and ripe plantain peels are rich source of minerals and phytochemicals. In this regard, their disposal or disuse must abate; because they could be nutritionally studied and well processed as a good source of nutrients, and even further, for biogas production. The presence of phytochemicals also demonstrates its potential to serve as nutraceuticals and a medicinally vital material in animal health.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

- Agama-Acevedo E, Sanudo-Barajas JA, Velez De La Rocha R, Gonzalez-Aguilar GA, Bello-Perez LA (2015). Potential of plantain peels flour (*Musa Paradisiaca*) as a source of dietary fibre and antioxidant compound. *Journal of Food* 14(1):117-123.
- Ajasin FO, Omole AJ, Oluokun JA, Obi OO, Owosibo A (2006). Performance characteristics of weaned rabbits fed plantain peel as replacement maize. *World Journal of Zoology* 1(1):30-32.
- Asquith TN, Butler LG (1986). Interaction of condensed tannins with selected proteins. *Phytochemistry* 25:1591-1593.
- Babatunde GM (1992). Availability of banana and plantain products for animal feeding. Machin D, Nyvold S (ed.), *Roots, tubers, plantains and bananas in animal feeding*. Roma: FAO. pp. 251-276.
- Babayemi JO, Dauda KT, Nwude DO, Ajiboye JA, Essien ER, Abiona OO (2010). Determination of potash alkali and metal contents of ashes obtained from peels of some varieties of Nigerian grown *Musa* species. *BioResources* 5(3):1384-1392.
- Bamhard UK (1985). *Physicochemical studies of African medicinal plants*. Bayero University Kano, Library. pp. 69-72.
- Betiku E, Sheriff OA (2014). Modeling and optimization of *Thevetia peruviana* (yellow oleander) oil biodiesel synthesis via *Musa paradisiacal* (plantain) peels as heterogeneous base catalyst: A case of artificial neural network vs. response surface methodology. *Industrial Crops and Products* 53:314-322.
- Bonire JJ, Jalil NSN, Lori JA (1990). Sodium and potassium content of two cultivars of white yam (*Dioscorea rotundata*) and their source soils. *Journal of the Science of Food and Agriculture* 53(2):271-274.
- Calles AH, Clavigo HE, Mane IH (2000). Ripe Pplantain *Musa paradisiaca* as energy source for growing pigs. In: *Effects of graded levels of ripe and unripe plantain peel meals on performance, organ weights and blood parameters of broiler chickens by Oyediji JO, Olomu JM, Godspower SA, Ojero OV (eds.)*. Nigerian Journal of Agriculture Food and Environment 11(4):28-33.
- Chen XH, Xia LX, Zhou HB, Qiu GZ (2010). Chemical composition and antioxidant activities of *Russula griseocarnosa* sp. *Journal of Agricultural and Food Chemistry* 58(11):6966-6971.
- Earl ZK, Warren MS (1961). Chemical composition of plant tissues. In: *Handbook of Biochemistry*, Red Wood Press. P 198.
- Eleazu CO, Iroaganachi M, Eleazu KC (2013). Ameliorative potentials of cocoyam (*Colocasia esculenta* L) and unripe plantain (*Musa paradisiaca*) on the relative tissue weight of streptozotocin induced diabetic rats. *Journal of Diabetes Research* 2013:1-8.
- Enontiemonria EV, Abiodun OJ, Eric KE (2016). Extraction and Use of Potassium Hydroxide from Ripe Plantain Peels Ash for Biodiesel Production. *Journal of Biobased Materials and Bioenergy* 10(1):1-9.
- Felgis F (1975). Anthraquinone stop test in organic analysis. *Elvesier Press, Amsterdam*. pp. 242-252.
- Food and Agriculture Organization (FAO) (2005). *Production year book for 2005, FAOSTAT Data*, Food and Agriculture Organization of the United Nations, Rome.
- Ighodaro OM (2012). Evaluation study on Nigerian species of *Musa paradisiaca* peels: phytochemical screening, proximate analysis, mineral composition and antimicrobial activities. *Researcher* 4(8):17-20.
- Ighodaro OM, Mairiga JP, Adeyi AO (2009). Reducing and anti-oxidant profiles of flavonoids in *ocimum gratissimum*. *International Journal of Chemical Sciences* 2:85-89.
- Israel AU, Akpan IA (2016). Mineral composition of ashed and charred palm (*Elaeis guineensis*) bunch and plantain (*Musa Pradisiaca*) Peel. *British Journal of Applied Science and Technology* 16(5):1-9.
- Jarup L (2003). Hazards of heavy metal contamination. *British Medical Bulletin* 68(1):167-182.
- Koleva II, Van Beek TA, Linssen JPH, de Groot A, Evstatieva, LN (2002). Screening of plant extract for antioxidant activity: A comparative study on three testing methods. *Phytochemical Analysis* 13(1):8-17.
- Middleton EJ, Kandaswami C (1992). Effects of flavonoids on immune and inflammatory cell function. *Biochemical Pharmacology* 43(6):1167-1179.
- Okorie DO, Eleazu CO, Nwosu P (2015). Nutrient and Heavy Metal Composition of Plantain (*Musa paradisiaca*) and Banana (*Musa paradisiaca*) Peels. *Journal of Nutrition and Food Sciences* 5(3):370.
- O'Connell B (2011). Select Vitamins and minerals in the management of diabetes. *Diabetes Spectrum* 14:133-148.
- Ojo OO, Nadro MS, Tella IO (2006). Protection of rats by extracts of some common Nigerian trees against acetaminophen induced hepatotoxicity. *African Journal of Biotechnology* 5(9):755-760.
- Onyegbado CO, Iyagba ET, Offor OJ (2002). Solid soap production using plantain peels ash as source of alkali. *Journal of Applied Sciences and Environmental Management* 6(1):73-77.
- Osborn DR, Voogt P (1978). *The analysis of nutrients in food*. Academic Press London. pp. 166-182.
- Soetan KO (2008). Pharmacological and other beneficial effects of Anti-nutritional factors in plants – A review. *African Journal of Biotechnology* 7(5):4713-4721.
- Sofowora A (1979). *Phytochemical methods, screening plants for bioactive agents*. Spectrum Books Ltd, Ibadan, Nigeria. pp. 134-156.
- Sofowora A (1993). *Medicinal Plants and Traditional Medicine in Africa*. Spectrum Books Ltd, Ibadan, Nigeria. pp. 343-383.
- Tchobanoglous G, Theisen H, Vigil S (1993). *Integrated Solid Waste Management: Engineering Principles and Management Issues*. McGraw- Hill, New York. pp. 3-22.
- Tisato F, Marzano C, Porchia M, Pellei M, Santinic C (2010). Copper in diseases and treatments, and copper-based anti-cancer strategies. *Medical Research* 30:708-749.

Wijetunge WMANK, Perera BGK (2016). Preparation of medicinal soap using leaf extract a *Punica granatum* (POMEAGRANATE). International Journal of Pharmacy and Biological Sciences 6(2):7-16.

Wolfe K, Wu X, Liu RH (2003). Antioxidants activity of apple peels. Journal of Agriculture and Food Chemistry 51:609-614.

Zenk MH (1996). Heavy metal detoxification in higher plants – a review. Gene 179:21-30.

Review

Inactivation of microbes by ozone in the food industry: A review

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Ozone is active against a broad spectrum of microorganisms. Ozone treatment can enhance safety and increase shelf life with limited impact on product quality. Ozone is known to be one of the strongest oxidizers that can have applications in foods. In the gaseous state, ozone is denser than air, colorless at lower concentrations and possesses a distinct odor. Ozone can be generated using a few methods, by photochemical procedures specifically UV light, electrolysis of water, with corona discharge being the most common method. In the food processing industry, ozone acts as a powerful sterilizer against both Gram-positive and Gram-negative bacteria, bacterial spores, fungi, viruses, and protozoa. Ozone affects the unsaturated lipids in the cell membrane causing leakage of cellular components that can lead to cell death. There are numerous examples to show that ozone has been successfully applied in food processing, specifically in sanitation by disinfecting food plant equipment and contact surfaces, packaging materials, water, air in storage and refrigeration systems, and for foods such as dried and fresh fruits and vegetables. The shelf life and quality of different food products can be maintained using ozone through reduction of spoilage microorganisms.

Key words: Ozone, food, inactivation, sanitation, bacteria.

INTRODUCTION

Ozone has been widely used in numerous industries including the food industry for many years (Fundo et al., 2018). Recently, there has been a renewed interest in ozone and its application in the food processing industry for use as a sanitizer (Lelieveld et al., 2016). Ozone or triatomic oxygen (O₃) is an unstable allotrope of oxygen. It is an effective alternative to chlorine compounds in the food industry (Ziyaina and Al Zogne, 2010). This is due to the advantage that, when decomposed into free

radicals, ozone leaves no residual components on the food product when decomposition is complete, liberating the main product of oxygen (Segat et al., 2014). Ozone use was approved by the US Food and Drug Administration US in 1982 for use in water treatment. In 2001, the FDA officially approved ozone for applications in the food industry and for direct contact with food products, including use as an antimicrobial and surface sanitizer in CIP (Clean-In-Place) systems and in fish,

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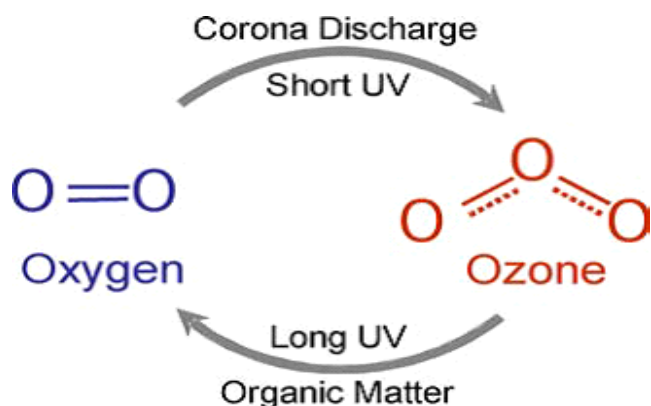


Figure 1. Formation of ozone by UV light (Wavelength shorter than 242.4 nm)

meat, and poultry processing plants (Al-Qadiri et al., 2019; Fundo et al., 2018; Najafi and Khodaparast, 2009; Zhang et al., 2016). There are several other applications of ozone in food processing, such as plant and equipment sanitation at food factories, surface hygiene, drinking water purification, and treatment for reuse of wastewater. Ozone can be applied to dried and fresh fruits and vegetables as a disinfectant (Rojas-Valencia, 2011). In food processing, ozone is used as a sterilizer against a wide range of microbes. Ozone is a triatomic allotrope of oxygen that is characterized by a high oxidation potential (Khadre et al., 2001; Lelieveld et al., 2016). Due to its antimicrobial properties, ozone conveys bactericidal properties that kill microorganisms such as viruses, bacteria, fungi and protozoa, which are causes of foodborne illness and food spoilage. Ozone actively destroys bacterial and fungal spores (Guzel-Seydim et al., 2004; Öztekin et al., 2006). Ozone treatment can remove up to 99% of bacteria and viruses at a concentration of 10 mg/l within 10 min.

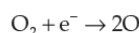
Ozone is more stable in its gaseous phase than its liquid phase. Gaseous ozone dissolves easily in water and has higher solubility than nitrogen (N₂) and oxygen (O₂) gases. Ozone solubility in cool water is 13 times more than that of oxygen at 0–30°C. Gaseous ozone is more soluble in water compared to oxygen and nitrogen, but less soluble than chlorine and carbon dioxide (CO₂) (Guzel-Seydim et al., 2004). Solubility and stability of ozone in water depends upon several physical parameters, such as water temperature (the solubility of ozone in water is higher at lower temperatures), ozone concentration, other materials in the water, pH, metal ions, radical scavengers, and use of mechanical stirring (Kim et al., 2003; O'Donnell et al., 2012).

The use of ozone in food plants has led researchers to further explore other applications within their facilities and to study its effect on microbes. Continued research is needed for relevant and concise information about this sanitizer. This review surveys information about this important topic.

OZONE GENERATION

There are two methods commonly used to produce ozone for food applications, corona discharge (CD) commercially and photochemically with ultraviolet (UV) light. Electrochemical, chemical, biochemical, thermal, and chemonuclear are other possibilities (Cullen et al., 2011; Guzel-Seydim et al., 2004; O'Donnell et al., 2012).

To generate ozone by the corona discharge method, oxygen molecules are passed through the electrical field where they are split, resulting in oxygen free radicals. The free radicals can react with diatomic oxygen to form a triatomic ozone molecule (O₃). To break the bond between the oxygen molecules (O–O) requires a great deal of energy. Formation of ozone can be represented in the following equations:



UV wavelength (188 μm) can be coupled with corona discharge to initiate the formation of oxygen free radicals through photo disassociation. In the photo disassociation process, a small percentage of oxygen molecules are split by UV rays, into unstable oxygen radicals. To become more chemically stable, O₁ radicals readily attach to surrounding O₂ molecules forming ozone shown in Figure 1 (Guzel-Seydim et al., 2004).

ADVANTAGES OF OZONE USAGE

One of the primary advantages of ozone is that it is a non-thermal process. It is a potent sanitizer and relatively safe and reliable for food processing applications. It has an oxidation potential of 2.07 V, which is much higher than chlorine (1.36 V) (Aguayo et al., 2006) while leaving no residue on food (Lelieveld et al., 2016; O'Donnell et al., 2012). Ozone half-life in water at room temperature is about 20 min, with it decomposing into molecular oxygen and posing few safety concerns about the consumption of residual ozone in a treated food. Because it rapidly decomposes into oxygen, there are no traces of toxic halogenated compounds present as residues (Isikber and Athanassiou, 2015). Ozone gas can be produced on-site, and it does not need to be stored or transported from another location; however, it must be used in a well-ventilated area since it is toxic when inhaled. However, a major disadvantage involves monitoring since as the lack of stable residue limits the ability for online testing (Cullen et al., 2011; Lelieveld et al., 2016; O'Donnell et al., 2012).

INACTIVATION MICROORGANISMS BY OZONE

Recent research points out that ozone is a powerful broad-spectrum antimicrobial agent, active against bacteria (Gram-positive and Gram-negative bacteria, bacterial spores, fungi, viruses, and protozoa.

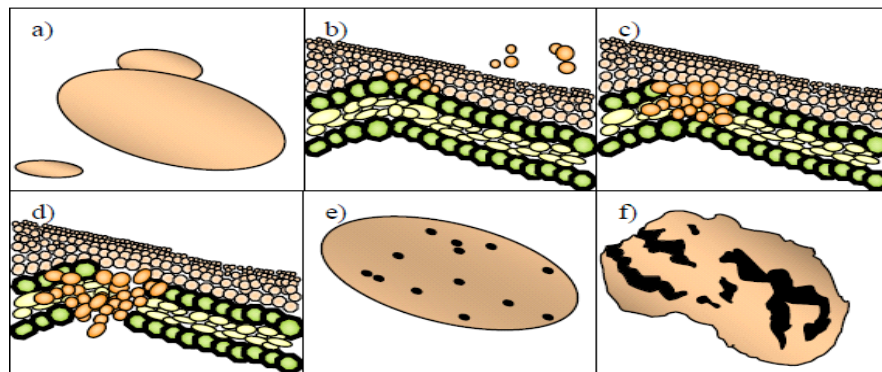


Figure 2. Bacteria undergoing lysis during disinfection with ozone. (A) the cell membrane is the first site under attack; then (B) the ozone attacks glycoproteins, glycolipids, or certain amino acids, and acts on the sulfhydryl groups of certain enzymes; (C) the effect of ozone on the cell wall begins to become apparent; (D) the bacterial cell begins to break down after coming in contact with ozone; E) the cell membrane is perforated during this process; and finally, (F) the cell disintegrates or suffers cellular lysis.

Source: Adapted from Rojas-Valencia (2011).

A deactivation involves destroying biological activities of a microbial cell such as inducing changes to structural components of the cell causing cell death through a change in cell permeability and cell lysis and by altering the ability of a cell to divide and thereby reproduce. Ozone damage results in breakage of the cellular membrane, inhibiting cellular reactivation mechanisms, and oxidizing unsaturated fatty acids, lipid fatty acids, glycoproteins, glycolipids, amino acids, sulfhydryl groups of certain enzymes, phenolic rings, and nucleic acids (Khadre et al., 2001).

The efficacy of ozone has been demonstrated against Gram positive *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus*, and Gram-negative *Pseudomonas aeruginosa* and *Yersinia enterocolitica*; *Escherichia coli* is one of the most sensitive to ozone damage, while Gram-positive cocci (*Staphylococcus* and *Streptococcus*) and Gram-positive bacilli (*Bacillus*) and mycobacteria are among the most resistant to ozone damage (Le Chevallier, Au, and World Health, 2004).

Ozone is a potent oxidant, as shown in Figure 2, and destroys microorganisms by reacting with oxidizable cellular components, particularly those containing double bonds.

The rate and degree of bacterial inactivation resulting from ozone treatment will depend upon the species. Findings from studies on ozone microbial inactivation are subsequently presented in the paper.

Effectiveness of ozone on various food products

The effect of ozone on bacteria depends on various factors, including the type of food product, microbial load,

concentration of ozone and a suitable ozone treatment system, and treatment time. Optimizing these factors can decrease damage to susceptible food products and lead to favorable outcomes in the majority of commodities. A 2007 study by Bialka and Demirci, documented treatment of blueberries and spinach with 5% (wt/wt) gaseous ozone (Bialka et al., 2008). They reported that ozone resulted in a 2.2 and 1.9 log, respectively, reduction of *Escherichia coli* O157:H7. Perry and Yousef (2011) conducted a research study exposing whole apples to 23–30 mg/L aqueous ozone, finding a reduction of about 3.7-log in *E. coli* O157:H7. Also, they noted that treatment of blueberries with 5% (wt/wt) gaseous ozone for one hour caused a 2.2-log reduction of *E. coli* O157:H7 (Perry and Yousef, 2011). Akbas and Ozdemir (2006) reported cell damage to *E. coli* exposed to 0.167/mg/min/L ozone, at different times, caused deformation, surface roughness and surface destruction within 60 min. A 90-min treatment caused cell rupture and cellular lysis as shown in Figure 3.

The pH plays a significant role in ozone inactivation. For example, lower pH values provide a higher inactivation rate. Based on this factor, it was revealed that ozone treatment concentration 0.048 mg/min/mL and pH 3 and pH 5 reduced about 5-log of *E. coli* in apple juice in 4- and 18-min treatments.

Ozone has significant results in preservation efficacy and has been evaluated in a variety of liquid food products, including milk and water (Kim et al., 1999; Kim et al., 2003). Selma et al. (2007) found that ozone treatment by 1.6 and 2.2 ppm for 1 min decreased *Shigella sonnei* populations in water by 3.7- and 5.6-log CFU/ml, respectively; moreover, ozone applied to skim milk effectively decreased psychrotrophic counts by 2.4-logs (5-35 mg/l, 5-25 min (Rojek, 1996; Selma et al.,

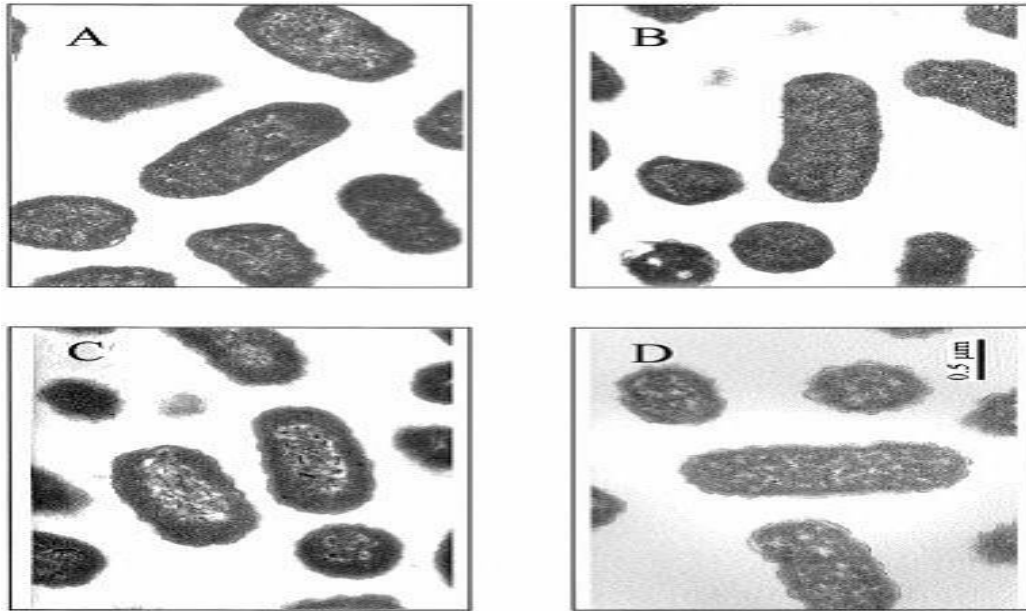


Figure 3. Transmission electron micrographs of *E. coli* cells after exposure to ozone in a continuous flow tubular reactor. Operating conditions: pH 7.2; temperature=208°C; No=5_109 CFU/l; t=30 s; co= (A) 0 mg/l, (B) 9 mg/l, (C) 18 mg/l and (D) 196 mg/l. Source: Adapted from Akbas and Ozdemir (2006) and Hunt and Mariñas (1999).

2007). Treatment of whey and apple juice also produced favorable microbial reduction. Greene et al. (1993) reported that ozone treatment at 0.5 ppm for 10 min eliminated more than 99% of the population of milk spoilage bacteria, such as *Pseudomonas fluorescens* and *Alcaligenes faecalis*.

A study reported ozone inactivation of Gram-positive bacteria such as *Streptococcus faecalis*, *S. aureus* and the yeast *Candida albicans*. One study found the *S. aureus* isolate to be more resistant than *S. faecalis* or *C. albicans*, and the longest time required to achieve total inactivation was 10 min. Exposure of *P. fluorescens*, *E. coli* O157:H7, *L. mesenteroides*, and *L. monocytogenes* to ozone at 2.5 ppm within less than a minute resulted in 5- to 6-log decrease for those bacteria (Cullen et al., 2011; Zhang et al., 1993).

Exposure to ozone (0.23–0.26 mg/L; 1.67 min; 24°C) achieved a 4.3 log inactivation of *Salmonella* Typhimurium. Ozone plus heat treatment is effective in causing greater inactivation at higher temperatures (50°C). According to a 2012 study, a higher process temperature (50°C) resulted in slightly higher inactivation (4.8 log in apple cider and orange juice) of *Salmonella* compared to 4.5 logs at 4°C due to higher ozone reactivity (Mukhopadhyay and Ramaswamy, 2012). Similarly, another study observed that apple cider treated with ozone can result in a 6-log reduction of *E. coli* O157:H7 following a 45 min ozone treatment (9 g h⁻¹) to apple cider held at 50°C. The same conditions produced a 4.8-log reduction of *Salmonella* within 30 min (Perry and Yousef, 2011).

Ozone gas could also be used in activating *Salmonella* on food surfaces. The experimental process involves applying ozone gas to inoculated fruits, resulting in maximum reductions of 1.5 and 0.9 log CFU/g. Treatment of ozone gas under pressure yielded greater reductions in *Salmonella* sp. in raspberries (1.9 CFU/g for *Salmonella* and *E. coli* O157:H7 2.8 log CFU/g) (Sung et al., 2014; Williams et al., 2005).

Viruses

Generally, viruses are more resistant to ozone compared to vegetative bacteria (Rojas-Valencia, 2011). Burleson et al. (1975) found that ozone killed viruses. In their waste-water treatment study, they found that ozone treatment successfully inactivated vesicular stomatitis virus, encephalomyocarditis virus, and GDVII virus after 15 s of treatment (Burleson et al., 1975). O'Donnell (2012) reported that viruses that contained lipid encirclement (lipid bodies) were more resistant to ozone than those that did not possess this feature. Also, he indicated that hepatitis A virus could be inactivated within 5 s by 0.4 ppm aqueous ozone treatment dose. Ozone can be effective in controlling Norwalk virus found in drinking water, with ozone (0.37 ppm) at pH 7 for 5 min at 5°C being effective reducing concentrations by greater than 3 logs in 10 s (O'Donnell et al., 2012).

The mechanic effect of ozone on viruses breaks the protein capsid into subunits, liberating RNA and disrupting virus adsorption to the host pili. In addition, ozone can randomly destroy the head, collar, contractile

sheath, endplate, and tail fibers and liberate the DNA from the head.

Fungi

Many fungi produce toxins that can cause foodborne illness. One genus, with the members *Aspergillus flavus* and *Aspergillus parasiticus*, produce aflatoxin that can cause illness in humans and animals' while other fungi can cause food spoilage. Ozone can deactivate fungi by causing irreversible cellular damage (Valencia, 2011) and by oxidizing mold toxins. Zorlugenç et al. (2008) found that when dried figs were exposed to gaseous ozone, aflatoxin B1 content in the figs was reduced by exposure to 13.8 mg L⁻¹ ozone gas at 15 and 30 min, and ozone treatment at 15 min was sufficient for deactivation of *A. flavus* and *A. parasiticus* cells. Also, they noted that the samples of dried figs that were artificially contaminated with aflatoxin when treated with gaseous ozone and ozonized water for 30, 60 and 180 min, experienced a degradation of aflatoxin B1 that increased with increasing ozonation time (Öztekin et al., 2006; Zorlugenç et al., 2008).

APPLICATIONS OF OZONE IN THE FOOD INDUSTRY

Ozone improves food preservation in an aqueous solution or as a gas because of its ability to disinfect food, food surfaces and contact surfaces within a food processing facility. Ozone can be used to sanitize process water and to treat the atmosphere within a food processing facility; for example, a storage unit. Usually, gaseous ozone is used inside an enclosed treatment chamber because inhalation is dangerous. Ozone has been used in sanitizing eggs, fresh fruits and vegetables, fresh fish, and in cold storage applications. Ozone can also be used for sanitizing packaging materials (Khadre et al., 2001; Kim et al., 2003).

Ozone applications in the dairy industry

Recent studies have examined using ozone to remove biofilms from food plant equipment. Biofilms are a source of microbial contamination in addition to causing reduced heat transfer (O'Donnell et al., 2012). Concerns with *E. coli* and *L. monocytogenes* contamination in processed dairy food products (O'Donnell et al., 2012) resulting from contaminated food contact surfaces has led to a number of interesting studies. In a study from Baumann et al. (2009), *L. monocytogenes* biofilms formed on stainless steel chips were deactivated by ozone treatment, and higher concentrations of ozone had a synergistic effect when used with sonication for a 60-s exposure time (Baumann et al., 2009). Another research study from Dosti et al. (2005) showed the effectiveness of ozone (0.6 ppm at 10 min) on sessile cells in a bacterial biofilm.

They observed that ozone significantly reduced biofilms formation by *Pseudomonas* spp. on stainless steel and recommended the use of ozone as an alternative to chlorine when sanitizing dairy processing equipment (Dosti et al., 2005). A common problem in cheese processing plants is mold growth that occurs when cheese is ripening in storage rooms. Thus, cheese becomes moldy and could become contaminated with Aflatoxin M1. Ozone has been used to control mold growth associated with cheese ripening rooms (O'Donnell et al., 2012).

Ozone applications in raw poultry and meats

A significant number of cases of food poisoning in humans occur from contaminated raw poultry and meat products with pathogenic bacteria such *L. monocytogenes*, *Campylobacter* spp., *Salmonella* spp. and other enteric bacteria. Contamination of meat and poultry also dictates its shelf-life (Khubaib, 2019). Ozone is useful for decontamination of beef. Kim et al. (2003) reported that gaseous ozone was effective in preventing growth of microorganisms on meat surfaces. In addition, ozone has demonstrated microbiocidal efficacy and safety when washing poultry carcasses (Kim et al., 2003).

Another application of ozone is to control *Salmonella enterica* in shell eggs, where ozone can be used at low temperatures and under mild pressure for cold sanitization treatments. Yousef and Rodriguez-Romo (2008) noted that treating *Salmonella* in eggs with gaseous ozone for 10 min at 22-25°C and 15 psi led to decreased growth of *Salmonella* populations by more than 5 log (Perry et al., 2008).

Ozone applications for vegetables

Vegetables prior to harvest can become contaminated for a number of reasons from exposure to dirty irrigation water, lack of employee hygiene, improper handling, and after-harvest storage, use of contaminated water when washing or cleaning, dirty processing equipment, or from insanitary handling at the transportation facilities. In addition, when cutting fresh vegetables, the loss of surface integrity could lead to penetration and rapid growth of microorganisms within plant tissue. Using ozone to sanitize fresh produce is known to work very well. In one study on contaminants in fresh lettuce treated by ozone (1.3 mM) mesophilic and psychrotrophic bacterial counts were reduced 3.9 and 4.6 log units, respectively, during 5 min of ozone treatment. Ozone treatment of alfalfa sprouts for 5 min decreased their microbial load by 1.2 log units.

In another study on treatment of alfalfa sprouts, sprouts were placed in ozonized water (30-32 ppm) and stirred for 20 min; the average count decreased to 4.8 × 10⁷CFU g⁻¹ and the sprouts had better quality and

Table 1. Summary of food decontamination studies by ozone.

Food Type	Treatment (Type, Concentration, Time)	Targeted microorganisms	Inference	Reference
Apple	Aqueous ozone (23–30 mg/L, 3 min)	<i>Escherichia coli</i> O157:H7	Up to 3.7 log reduction on surface, 1 log reduction in stem-calyx	Perry et al. (2008)
Blueberry	Gaseous ozone (5%, wt/wt, 64 min)	<i>E. coli</i> O157:H7 <i>Salmonella enterica</i>	2.2 log reduction 1.0 log reduction	Bialka and Demirci (2007, 2008)
Spinach	In-package gaseous ozone (1.6 mg/L for air 4.3 mg/L for oxygen gas, 5 min) and with 5% (wt/wt) gaseous ozone	<i>E. coli</i> O157:H7	Up to 5 log reduction	Bialka and Demirci (2007) and Bialka et al. (2008)
Red pepper flake	Gaseous ozone (9 ppm, 360 min)	<i>Bacillus cereus</i> spores	1.5 log reduction	Akbas and Ozdemir (2008) and Asill et al. (2013)
Fabricated beef surfaces	Aqueous ozone (5 ppm) followed by heating (55°C)	<i>Clostridium perfringens</i> spores	2.09 log reduction for vegetative cells 0.87 log reduction for spores	Novak and Yuan (2004) and Pohlman (2012)
Alfalfa seeds	Aqueous ozone (21.8 ppm, 10–20 min) followed by heating (60°C, 3 h)	<i>Listeria monocytogenes</i>	1.48 log ₁₀ CFU/g	Wade et al. (2003)
Table grapes	Gaseous ozone (5000 µL/L, up to 60 min)	<i>Botrytis cinerea</i> (gray mold)	50–60% reduction	Ozkan et al. (2011)
Dried figs	Gaseous ozone (13.8 mg/L, 180 min)	Aflatoxin B1	95% reduction	Oner and Demirci (2016)
Corn kernels	Gaseous ozone (10–12%, wt/wt, 96 h)	Aflatoxin	92% reduction	Oner and Demirci (2016)
Potato strips	Heating (60°C for 10 min, 100°C for 5 min) followed by in-package gaseous ozone (5%, wt/wt, 15–30 min)	Mesophilic Psychrotrophic Mold-yeast	No growth up to 4 weeks at 4°C	Oner et al. (2011)

Source: Lelieveld et al. (2016).

texture (Kim et al., 2003). Other applications of ozone are described as follows:

- i) Removal of pesticide residues from drinking and wastewater
- ii) Disinfection of drinking and wastewater
- iii) Control of air pollution
- iv) Sterilization of containers and food storage tanks
- v) Disinfection of aquaculture farms
- vi) Sterilization of bottles and cans
- vii) Disinfection of drinking and wastewater
- viii) Organic oxidation of drinking and wastewater.

OZONE - WORKER SAFETY ISSUES

Ozone may cause headaches and irritation of

eyes, upper respiratory tract, and lungs at concentration used for food treatment, but usually these effects disappear after breathing fresh air. According to the American Council of Industrial Hygienists, the short-term exposure limit is 0.3 ppm for 15 min. However, Occupational Safety and Health Administration (OSHA) regulations state that ozone concentration of 0.1 ppm inhaled during an eight-hour workday is the maximum limit (O'Donnell et al., 2012).

OZONE AND REGULATORY RESTRICTIONS

Ozone has been 'Generally Recognized as Safe' (GRAS) in food processing since 1997 in the U.S. (Patil et al., 2009). Furthermore, in 2001, the Food and Drug Administration (FDA) approved ozone

as an antimicrobial agent for food additive applications (O'Donnell et al., 2012; Rice, 2011). Studies have been proved that ozone has effects significantly on a wide range of microorganisms including bacterial and spores, viruses, fungi, and parasites, with different concentrations and times. The application and effect of ozone on food decontamination are summarized in Table 1.

CONCLUSION

More attention is now focused on ozone as a powerful sanitizer that may meet the expectations of food industry. Ozone has been proven to be a powerful disinfectant and antimicrobial agent, reacting with intracellular enzymes' nucleic material and its cell envelope. It is suitable for application

in the food industry, where it has been used in a gaseous or an aqueous form.

The food industry is currently in need of innovative processing technologies in order to meet consumer demand of fresher, safe products. Ozone might be considered as an emerging or best available alternative technology in food storage and in cleaning and disinfection of closed equipment in the food milk and drink industry. Examples of the use of ozone in food industry include use of ozone as disinfectant for meat, vegetables, fruits, and drinking water, and for its use in storing food in tanks for many years. It has also been used in reuse of wastewater treatment and lowering biochemical oxygen demand (BOD) and chemical oxygen demand (COD). Recent studies have also investigated using ozone to remove biofilms from food plant equipment.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Aguayo E, Escalona V, Artés F (2006). Effect of cyclic exposure to ozone gas on physicochemical, sensorial and microbial quality of whole and sliced tomatoes. *Postharvest Biology and Technology* 39(2):169-177.
- Akbas MY, Ozdemir M (2006). Effect of different ozone treatments on aflatoxin degradation and physicochemical properties of pistachios. *Journal of the Science of Food and Agriculture* 86(13): 2099-2104.
- Akbas MY, Ozdemir M (2008). Effect of gaseous ozone on microbial inactivation and sensory of flaked red peppers. *International Journal of Food Science and Technology*, 43(9), 1657-1662.
- Al-Qadiri HM, Smith S, Sialaff AC, Govindan BN, Ziyaina M, Al-Alami N, Rasco B (2019). Bactericidal activity of neutral electrolyzed water against *Bacillus cereus* and *Clostridium perfringens* in cell suspensions and artificially inoculated onto the surface of selected fresh produce and polypropylene cutting boards. *Food Control* 96:212-218.
- Asill RV, Azizi M, BAHREINI M, Arouiee H (2013). The investigation of decontamination effects of ozone gas on microbial load and essential oil of several medicinal plants. *Notulae Scientia Biologicae* 5(1):34-38.
- Baumann AR, Martin SE, Feng H (2009). Removal of *Listeria monocytogenes* biofilms from stainless steel by use of ultrasound and ozone. *Journal of Food Protection* 72(6):1306-1309.
- Bialka KL, Demirci A (2007). Decontamination of *Escherichia coli* O157: H7 and *Salmonella enterica* on blueberries using ozone and pulsed UV-light. *Journal of Food Science* 72(9):M391-M396.
- Bialka KL, Demirci A (2008). Decontamination of *Escherichia coli* O157: H7 and *Salmonella enterica* on blueberries using ozone and pulsed UV-light written for presentation at the. Paper presented at the 2008 Providence, Rhode Island, June 29–July 2, 2008.
- Bialka KL, Demirci A, Puri VM (2008). Modeling the inactivation of *Escherichia coli* O157: H7 and *Salmonella enterica* on raspberries and strawberries resulting from exposure to ozone or pulsed UV-light. *Journal of Food Engineering* 85(3):444-449.
- Burleson GR, Murray T, Pollard M (1975). Inactivation of viruses and bacteria by ozone, with and without sonication. *Applied Microbiology* 29(3):340-344.
- Cullen PJ, Tiwari BK, Valdramidis V (2011). Novel thermal and non-thermal technologies for fluid foods: Academic Press.
- Dosti B, Guzel-Seydim Z, Greene AK (2005). Effectiveness of ozone, heat and chlorine for destroying common food spoilage bacteria in synthetic media and biofilms. *International Journal of Dairy Technology* 58(1):19-24.
- Fundo JF, Miller FA, Tremarin A, Garcia E, Brandão TR, Silva CL (2018). Quality assessment of Cantaloupe melon juice under ozone processing. *Innovative Food Science and Emerging Technologies* 47:461-466.
- Guzel-Seydim ZB, Greene AK, Seydim A (2004). Use of ozone in the food industry. *LWT-Food Science and Technology* 37(4):453-460.
- Hunt NK, Mariñas BJ (1999). Inactivation of *Escherichia coli* with ozone: chemical and inactivation kinetics. *Water Research* 33(11):2633-2641.
- Isikber AA, Athanassiou CG (2015). The use of ozone gas for the control of insects and micro-organisms in stored products. *Journal of Stored Products Research* 64:139-145.
- Khadre M, Yousef A, Kim JG (2001). Microbiological aspects of ozone applications in food: a review. *Journal of Food Science* 66(9):1242-1252.
- Khubaib S (2019). Prevalence of *E. coli* in indigenous chicken meat; A Pakistan's perspective. *International Journal of Food and Allied Sciences* 4(1):18-27.
- Kim JG, Yousef AE, Dave S (1999). Application of ozone for enhancing the microbiological safety and quality of foods: a review. *Journal of Food Protection* 62(9):1071-1087.
- Kim JG, Yousef AE, Khadre MA (2003). Ozone and its current and future application in the food industry. *Advances in Food Science and Nutrition* 45:167-218
- Lelieveld HL, Holah J, Gabric D (2016). *Handbook of hygiene control in the food industry*: Woodhead Publishing.
- Mukhopadhyay S, Ramaswamy R (2012). Application of emerging technologies to control Salmonella in foods: A review. *Food Research International* 45(2):666-677.
- Najafi MBH, Khodaparast MH (2009). Efficacy of ozone to reduce microbial populations in date fruits. *Food control* 20(1):27-30.
- Novak JS, Yuan JT (2004). Increased inactivation of ozone-treated *Clostridium perfringens* vegetative cells and spores on fabricated beef surfaces using mild heat. *Journal of Food Protection* 67(2):342-346.
- O'Donnell C, Tiwari BK, Cullen P, Rice RG (2012). *Ozone in food processing*: John Wiley and Sons.
- Oner M, Demirci A (2016). Ozone for food decontamination: Theory and applications. In *Handbook of hygiene control in the food industry* (pp. 491-501): Elsevier.
- Oner ME, Walker PN, Demirci A (2011). Effect of in-package gaseous ozone treatment on shelf life of blanched potato strips during refrigerated storage. *International Journal of Food Science and Technology* 46(2):406-412.
- Ozkan R, Smilanick JL, Karabulut OA (2011). Toxicity of ozone gas to conidia of *Penicillium digitatum*, *Penicillium italicum* and *Botrytis cinerea* and control of gray mold on table grapes. *Postharvest Biology and Technology* 60(1):47-51.
- Öztekin S, Zorlugenç B, Zorlugenç FKI (2006). Effects of ozone treatment on microflora of dried figs. *Journal of Food Engineering* 75(3):396-399.
- Patil S, Bourke P, Frias JM, Tiwari BK, Cullen PJ (2009). Inactivation of *Escherichia coli* in orange juice using ozone. *Innovative Food Science and Emerging Technologies* 10(4):551-557.
- Perry J, Rodriguez-Romo L, Yousef A (2008). Inactivation of *Salmonella enterica* serovar enteritidis in shell eggs by sequential application of heat and ozone. *Letters in Applied Microbiology* 46(6):620-625.
- Perry JJ, Yousef AE (2011). Decontamination of raw foods using ozone-based sanitization techniques. *Annual review of Food Science and Technology* 2:281-298.
- Pohlman FW (2012). Ozone in meat processing. *Ozone in Food Processing*. pp. 123-136.
- Rojas-Valencia M (2011). Research on ozone application as disinfectant and action mechanisms on wastewater microorganisms. *Virus* 3(4.0).
- Rojek U, Hill A, Griffiths. M (1996). Preservation of milk by hyperbaric ozone processing. *Journal of Dairy Science* 78(Suppl.1):125.
- Segat A, Biasutti M, Iacumin L, Comi G, Baruzzi F, Carboni C, Innocente N (2014). Use of ozone in production chain of high

- moisture Mozzarella cheese. *LWT-Food Science and Technology* 55(2):513-520.
- Selma MV, Beltrán D, Allende A, Chacón-Vera E, Gil MI (2007). Elimination by ozone of *Shigella sonnei* in shredded lettuce and water. *Food Microbiology* 24(5):492-499.
- Sung HJ, Song WJ, Kim KP, Ryu S, Kang DH (2014). Combination effect of ozone and heat treatments for the inactivation of *Escherichia coli* O157: H7, *Salmonella Typhimurium*, and *Listeria monocytogenes* in apple juice. *International Journal of Food Microbiology* 171:147-153.
- Wade W, Scouten A, McWatters K, Wick R, Demirci A, Fett W, Beuchat, L (2003). Efficacy of ozone in killing *Listeria monocytogenes* on alfalfa seeds and sprouts and effects on sensory quality of sprouts. *Journal of Food Protection* 66(1):44-51.
- Williams RC, Sumner SS, Golden DA (2005). Inactivation of *Escherichia coli* O157: H7 and *Salmonella* in apple cider and orange juice treated with combinations of ozone, dimethyl dicarbonate, and hydrogen peroxide. *Journal of Food Science* 70(4):M197-M201.
- Zhang T, Xue Y, Li Z, Wang Y, Yang W, Xue C (2016). Effects of ozone on the removal of geosmin and the physicochemical properties of fish meat from bighead carp (*Hypophthalmichthys nobilis*). *Innovative Food Science and Emerging Technologies* 34:16-23.
- Zhang XJ, Xue G, Agarwal A, Tsu R, Hasan MA, Greene J, Rockett A (1993). Thermal desorption of ultraviolet–ozone oxidized Ge (001) for substrate cleaning. *Journal of Vacuum Science and Technology A: Vacuum, Surfaces, and Films* 11(5):2553-2561.
- Ziyaina M, Al Zogne O (2010). A study of effects of chlorine on bacteria pathogens in drinking water. WSTA 9th Gulf Water Conference Sultanate of Oman, Water Science and Technology Association. pp. 742-753
- Zorlugenç B, Zorlugenç FK, Öztekin S, Evliya IB (2008). The influence of gaseous ozone and ozonated water on microbial flora and degradation of aflatoxin B1 in dried figs. *Food and Chemical Toxicology* 46(12):3593-3597.

Full Length Research Paper

Impact of instant controlled pressure drop (DIC) treatment on the technological quality of gluten-free bread based on rice-field bean formula using design of experiments

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This work aimed to investigate the impact of Instant Controlled Pressure Drop (DIC) treatment on the technological quality of Gluten-Free Bread (GFB) based on Rice-Field bean Formula (RFF). Two DIC factors were used as independent parameters of a Design of Experiments (DoE); X_1 : DIC temperature ranged from 100 to 165°C which was rigorously correlated with the dry saturated steam pressure ranged between 0.1 and 0.7 MPa, and X_2 : the processing time ranged from 20 to 60 s. The main response (Y) was the specific Volume (Vsp) of the GFB. The statistical analysis was performed using the Response Surface Methodology (MSR) to study and optimize the DIC treatment effect on technological quality (Vsp) of the GFB. The breads crumb was characterized by image analysis. The GFB optimum from RFF was obtained with DIC steam pressure of 0.3 MPa (temperature of 132.5°C) for a short treatment time of 20 s. It provided the highest Vsp of $2.7 \pm 0.04 \text{ cm}^3/\text{g}$ with an improvement of 10.20% than the GFB control and a great total number of *alveoli*. Hence, DIC treatment was an effective improvement mean of technological quality of GFB based on RFF for celiac patients.

Key words: Instant controlled pressure drop (DIC), rice-field bean formula, gluten-free bread, design of experiments (DoE), specific volume, crumb structure.

INTRODUCTION

Celiac disease (CD) is considered as one of the most widespread gastro-intestinal diseases. It is a state of

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autoimmune response to gluten proteins in a genetically predisposed subject, characterized by nutriment malabsorption caused by the atrophy of intestinal mucosa (Lionetti and Catassi, 2011; Lebwohl et al., 2018). It touches approximately 1% of the world's population with a great variation between countries (Makharia et al., 2011; Do Nascimento et al., 2017). Currently, the only treatment for CD is exclusively dietetic: a lifetime strict gluten-free diet, which entails removing any source of gluten containing in wheat, barley and rye and to substitute them by other gluten-free cereals such as rice and corn (O'Shea et al., 2014).

Bread is the basic consumed food in the world. However, its manufacture based on gluten-free ingredients remains difficult with a lower quality than the conventional wheat bread (Benatallah et al., 2012), due to the absence of the gluten, which is a great challenge for cereal researchers on gluten-free bread making (Masure et al., 2016). Many studies carried out for answering this problem were based on substituting the gluten by adding specific ingredients and additives like starches (Bourekoua et al., 2016; Bourekoua et al., 2018), proteins (Phongthai et al., 2016), enzymes (Mohammadi et al., 2015; Calle et al., 2020) and hydrocolloids (Benatallah et al., 2012).

Furthermore, the National Council of Food (CNA) (2009) of France highlighted the interest of the use of physical treatments in food industry in which these treatments could modify the chemical, functional, molecular, and structural properties of the raw materials used in the production of various foods. Among these physical treatments, the Instant Controlled Pressure Drop (French: *Détente Instantanée Contrôlée*- DIC) technology, which was defined in 1988, as a high temperature-short time stage usually carried out by establishing high-pressure dry saturated steam-high temperature (usually between 0.1-0.7 MPa relating with 100-165°C) during 5-60 s. This ends by an instant pressure-drop towards a vacuum of about 0.005 MPa and an instant cooling, at a rate $\Delta P/\Delta t$ higher than 0.5 MPa s⁻¹ (Allaf and Vidal, 1989).

This thermo-mechanical treatment method has been used in many industrial applications such as drying and texturing of plants (Louka and Allaf, 2002; Albitar et al., 2011; Nguyen, 2015), microbiological decontamination (Setyoprato et al., 2009), extraction volatile compounds and essential oils (Kristiawan et al., 2004; Besombes et al., 2010), and steam treatment of cereals (Habba and Allaf, 1997; Duong et al., 2008; Pilatowski et al., 2010).

However, the effect of Instant Controlled Pressure Drop (DIC) treatment on the quality of GFB has never been previously studied. Therefore, this study aimed to investigate and optimize the effect of DIC treatment on the technological quality (specific volume) of GFB based on RFF, with the objective of improving the situation of celiac patients.

MATERIALS AND METHODS

Raw materials

White long rice grain (*Oryza sativa*) from Basmati variety obtained from India and field bean seeds (*Vicia faba minor*) obtained from an Egyptian Company (Elamir Company, Egypt) were used in our study. Rice and field bean seeds were ground by a laboratory mill (Moulinex, France); thereafter, the flours obtained were sieved manually with 200- μ m seive. The Gluten-Free Formula (GFF) used was based on the mixture of rice and field bean flour with a ratio 2/1 (w/w). The soft wheat flour was used for the control bread. Additional ingredients were used such as bakery freeze-dried yeast, *Saccharomyces cerevisiae* (S.I. Lesaffre, France) and salt (ENASel, Algeria). All these raw materials were purchased from an Algerian local market.

Chemical composition of flours

Chemical composition of rice and field bean flours was determined following the ISO methods for moisture (ISO 712: 2009) and ash content (ISO 2171: 2010), and the AACC methods (1995) for protein (AACC 46-10) and lipid content (AACC 30-10) in three replications. Total carbohydrates content was evaluated by subtracting the total percentage of the other components from one hundred.

Instant controlled pressure drop (DIC) treatment of gluten-free formula

In the present study, DIC equipment used for the GFF treatment was a laboratory scale DIC unit (manufactured and provided by ABCAR-DIC Process, La Rochelle, France) (Figure 1). It consists of 11-L processing vessel with heating jacket, where samples are set and treated, a 1.6 m³-vacuum tank with cooling water jacket, a water ring vacuum pump, a steam generator with a thermally isolated storage tank and a specific condensed steam trap; a large-section pneumatic valve that assures an "instant" connection between the vacuum tank and the processing vessel that can be opened in less than 40 ms (Allaf, 2013).

DIC treatment of the different GFF samples, starts by placing 180 g of sample within a bag of coffee-type filter and positioning it in the treatment vessel at atmospheric pressure (Figure 2-phase a). After closing it, an initial vacuum stage (about 5 kPa = 0.005 MPa) was established (Figure 2-phase b) in order to remove the air presented in the vessel and, thus to assure close contact between the surface of the sample and the saturated dry steam to be injected just after (Figure 2-phase c). As demonstrated by Allaf and Allaf (2014), the use of dry saturated steam allows a subsequent hugely-fast heating by condensation. In our case, dry saturated steam pressure ranged between 0.10 and 0.70 MPa according to a specific design of experiments (DoE) we adopted based on the know-how of the research team, the literature, and a preliminary series of trials. This allowed the treatment temperature to be between 100 and 165°C. This stage (Figure 2-phase d) of 20 to 60 s ends by an abrupt pressure-drop towards a vacuum (5 kPa = 0.005 MPa) (Figure 2-phase e) obtained by opening the pneumatic large-section instant valve. Finally, the atmospheric pressure was restored in the treatment vessel (Figure 2-phase g) and the sample was recovered.

Design of experiments (DoE)

In order to study the effect of DIC operating parameters on the Vsp of GFB based on RFF, a specific DoE was carried out. It was a 5-level (- α , -1, 0, +1, + α) DoE with two factors; X₁: DIC temperature

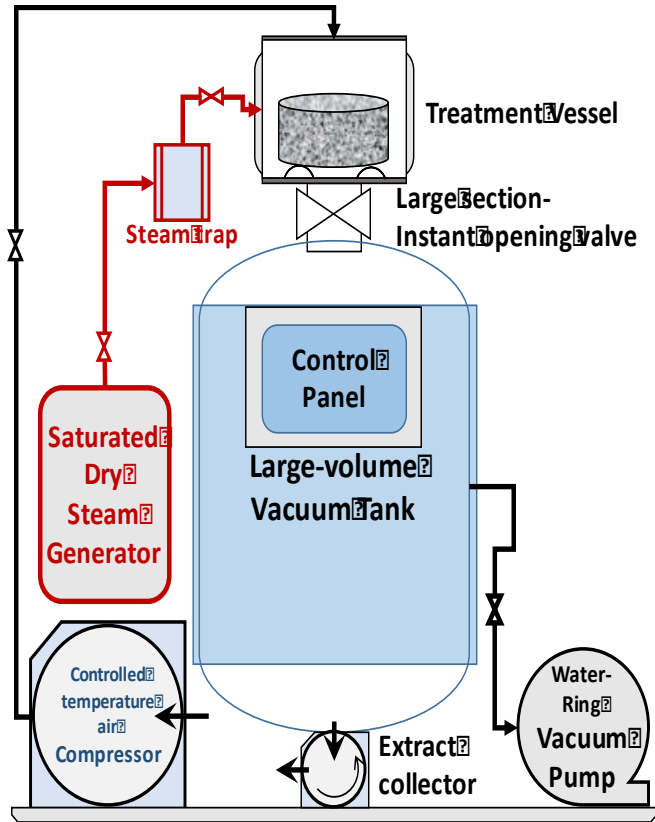


Figure 1. Schematic diagram of the Instant Controlled Pressure Drop (DIC) apparatus. Source: Allaf (2013).

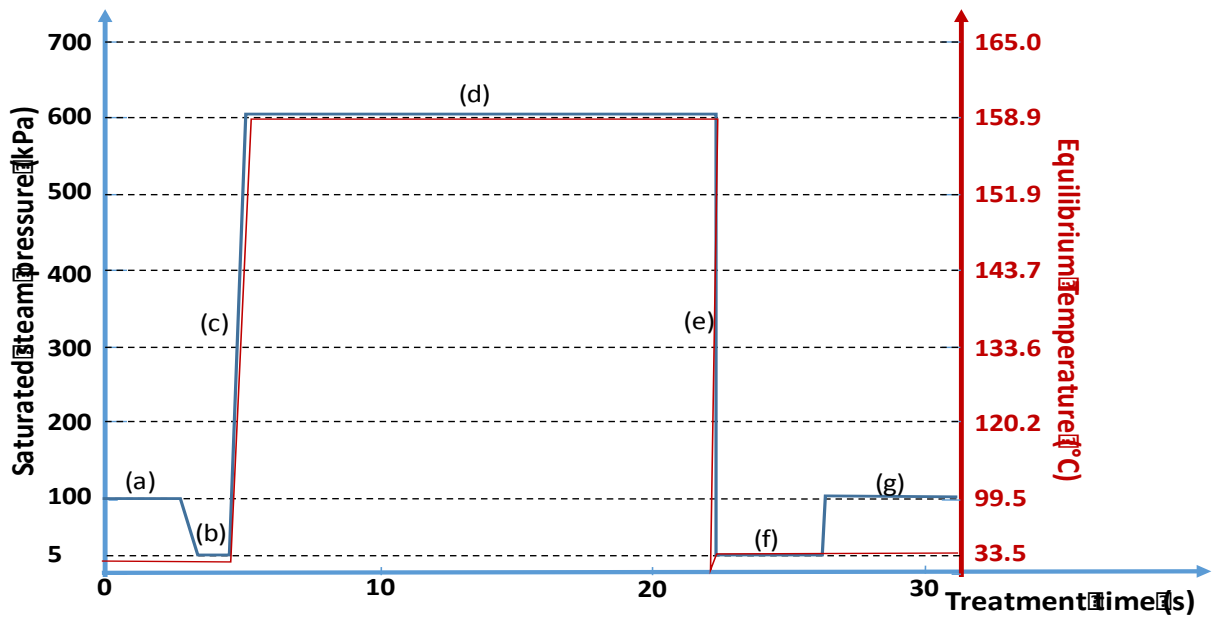


Figure 2. Temperature and pressure history of a DIC processing cycle: (a) Sample at atmospheric pressure; (b) Initial vacuum; (c) Saturated steam injection to reach the selected pressure and temperature; heating by condensation; (d) stage for almost homogenizing both temperature and water content within the product; (e) abrupt pressure-drop towards a vacuum of ~4-5 kPa coupled to an autovaporization; (f) vacuum of 4-5 kPa; (g) releasing to the atmospheric pressure. Source: Allaf (2013)

Table 1. Codes levels values of factors used in the Design of Experiments (DoE).

Code level		- α	-1	0	+1	+ α
X1: Temperature (T) coupled with the saturated dry steam pressure (P)	T (°C)	100.0	109.5	132.5	155.5	165.0
	P (MPa)	0.10	0.15	0.30	0.55	0.70
X2: Thermal treatment time (s)		20.0	25.9	40.0	54.1	60.0

Table 2. Run experimental values used in DIC treatment and added water to make dough of differently DIC treated rice-field bean formula.

Run	Coded variable	X1: Temperature (T) accorded to the steam pressure (P)		X2: Thermal treatment time (s)	Amount of water added (mL)
		T (°C)	P (MPa)		
1	(0, 0)	132.5	0.30	40	219.5
2	(+1.414, 0)	165.0	0.70	40	220.56
3	(0, +1.414)	132.5	0.30	60	221.9
4	(0, 0)	132.5	0.30	40	219.63
5	(+1, +1)	155.5	0.55	54	221.46
6	(+1, -1)	155.5	0.55	26	220.8
7	(0, 0)	132.5	0.30	40	221.03
8	(-1, -1)	109.5	0.15	26	219.93
9	(-1, +1)	109.5	0.15	54	219.83
10	(0, 0)	132.5	0.30	40	222.36
11	(-1.414, 0)	100.0	0.10	40	222.93
12	(0, -1.414)	132.5	0.30	20	222.5
13	(0, 0)	132.5	0.30	40	220.76

(T) coupled with the dry saturated steam pressure (P) and X₂: the thermal treatment time (t) (Table 1). In this case, DoE included 13 trials: 2⁰=2²=4 factorial points (-1,-1), (-1,+1), (+1,-1) and (+1,+1); 2×n=2×2=4 star-points (- α ,0), (0,- α), (0,+ α), and (+ α ,0); and 5 replicates for the center-point (0,0) were added. DIC treatment experiments were carried out in random using the operating conditions done in Table 2. The saturated dry steam pressure (expressed in Pa) applied in the current DIC range was accorded to the temperature level (T) (expressed in K) following the Equation 1:

$$P (Pa) = 10^{\left(10.09938 - \frac{1681}{T - 43.037}\right)} \quad (1)$$

The mathematical model used with the DoE is a second order model, which allows the study of the linear, quadratic and interaction effects according to the following Equation 2:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (2)$$

Where Y is response variable; X₁, X₂: are independent variables, treatment temperature and time, respectively; β_0 is the constant coefficient; β_1 and β_2 are regression linear coefficients; β_{11} and β_{22} are regression quadratic coefficients and β_{12} is interaction regression coefficient.

Optimization

The DIC treatment optimization consisted of defining the highest value of the studied response (V_{sp}). For that, the response surface of the V_{sp} of GFB from RFF was represented versus DIC factors (X₁ and X₂) of the couple (temperature-time). To confirm the optimized result computed by the MSR, a second experiment series

was performed including selected couples of temperature-time inside and outside the optimal delimited zone, in which the V_{sp} of the obtained GFB was determined.

Bread making process

In bread making test, the GFF used was based on rice and field bean flour with a ratio 2/1 (w/w) aimed to obtain a better nutritional balance of essential amino-acids (FAO, 1982; Benatallah et al., 2012).

For the 13 samples of treated DIC rice-field bean formula and the untreated formula, the amount of water added to each sample depended on its moisture content and it was calculated so that all the dough samples had the same final water content (70 g of water for 100 g of dough) (Table 2). So, preliminary tests allowed defining the hydration levels of manufacturing GFB as ranged between 219.5 and 222.93 mL of water for 100 g of rice/field bean flour. As for the control dough with soft wheat, preliminary tests based on obtaining the best V_{sp} of bread helped to retain an optimal hydration level of 61 mL of water for 100 g of wheat flour.

The bread recipe used consisted of 66.66 g of rice flour, 33.33 g of field bean flour, 2 g of salt, 2 g of instant freeze-dried yeast (*Saccharomyces cerevisiae*) and the amount of water suitable for each sample (Table 2). These ingredients were mixed for 15 min twice, with a 5-min break, in a kneader (HEIDOLPH RZR 2020, Germany) at 25°C. The resulting dough was divided in lumps (45 g) and put into a silicon mold (9×4.7×3 cm³), and then proofed at 37°C in wet atmosphere for 45 min in a fermentation cabinet (MEMMERT, France). The baking tests were performed in an electric oven (SAMSUNG, Germany) at 230°C in wet atmosphere for 55 min for GFB based on DIC-treated rice-field bean formula, 22 min for the control GFB and 15 min for the control wheat bread.

Table 3. Chemical composition of rice flour, field bean flour, and gluten free formula (d.b).

Flour	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrate ^a (%)
Rice flour (RF)	10.83% ± 0.01	6.37% ± 0.02	0.33% ± 0.01	0.59% ± 0.03	92.71
Field bean (FF)	10.54% ± 0.04	29.41% ± 0.03	1.99% ± 0.01	3.18% ± 0.02	65.42
RF/FF (2/1) ^b	10.73	14.05	0.88	1.45	83.61

^aCarbohydrates content estimated by difference; ^bResults obtained by calculation (2:1).

Table 4. Statistical parameters of specific volume of gluten-free bread of treated DIC rice-field bean formula (Empirical Statistical Model: $Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_{11}X_1^2 + \beta_{22}X_2^2 + \beta_{12}X_1X_2$).

Regression coefficient	β_0	β_1	β_2	β_{11}	β_{22}	β_{12}
Value	-0.48	0.0567	-0.0301	-0.000159	0.001068	-0.000562
P- value	0.000	0.117	0.033	0.469	0.093	0.248

Gluten-free bread quality evaluation

After 60 min post-baking, GFB characterization was carried out by the determination of Vsp and the image analysis of bread crumb structure.

Volume was determined by the rapeseed displacement method according to the AACC Approved Method 10.05 (AACC, 2000). Vsp of bread expressed in "cm³/g" was calculated by dividing its volume (V) by its mass (M).

According to Gonzales-Barron and Butler (2006), image analysis of bread crumb was performed using Image J software (version 1.43, National Institutes of Health, USA). Slices of bread (1 cm thick) were scanned using a flatbed scanner (Epson stylus SX 105) with a resolution of 300 dots per inch. The parameters recorded were the number of cells, the average surface and cells circularity (shape).

Statistical analysis

Statistical analysis of the design of experiments (DoE) was performed with MINITAB 17 software (Minitab Inc., PA State College, USA) and the response surface was traced using STATISTICA version 10 software (Stat Soft, France). The K-means test was applied to classify (regroup) the different types of breads. A coefficient of determination (R^2) was computed and the model adequacy was tested by separating the residual sum of squares into pure error and lack-of-fit. The significance level was set at 0.05.

RESULTS AND DISCUSSION

Chemical composition of raw materials

The chemical composition results of rice and field bean flour used, expressed in percentage (based on dry basis), are given in Table 3. Rice flour was characterized by high carbohydrates (92.71%), lower protein (6.37%), fat (0.33%) and ash (1.37%) contents compared to field bean flour. The calculation of GFF composition based on rice and field bean flour with a ratio 2/1 indicated improvement in protein (14.05%), fat (0.88%), and ash (1.45%) contents due to the incorporation of field bean

flour. The results obtained are in agreement with those reported for GFB made with rice/field bean flour with 2/1 (w/w) by Benatallah et al. (2012) and Bourekoua et al. (2016). Thus, the incorporation of legumes flours in gluten-free products enhances their nutritional quality (Lamacchia et al., 2014).

Model fitting

According to Goupy (2013) and Granato and de Araújo Calado (2014), the calculation of R^2 coefficient is used to measure the DoE empirical model quality and the lower lack-of-fit indicated the adequacy of the model. The statistical analysis of the effect of DIC operating parameters (X_1 : the steam temperature coupled with the pressure, and X_2 : the thermal treatment time) on the Vsp (Y) of GFB indicated that the fitting model was adequate due to the reasonable value of R^2 (0.73) and a non-significant lack-of-fit test (0.55).

Effect of DIC treatment on specific volume of gluten-free breads

The Vsp is one of the most important visual characteristics of bread, strongly influencing consumer choice, and it is the key-parameter for the bread quality evaluation (Hager and Arendt, 2013). The parameters of the statistical empirical model of the Vsp of GFB versus DIC factors are summarized in Table 4. It was observed that only the linear effect of DIC processing time X_2 was significant with the value 0.033, based on the p value relative to 0.050 ($p < 0.05$), whereas, the other terms were not significant ($p > 0.05$).

The effect of DIC operating parameters X_1 and X_2 on Vsp of GFB based on RFF is shown as response surface in Figure 3. It shows that the Vsp values varied from 1.2 to 2.8 cm³/g. Moreover, the highest Vsp was lower than

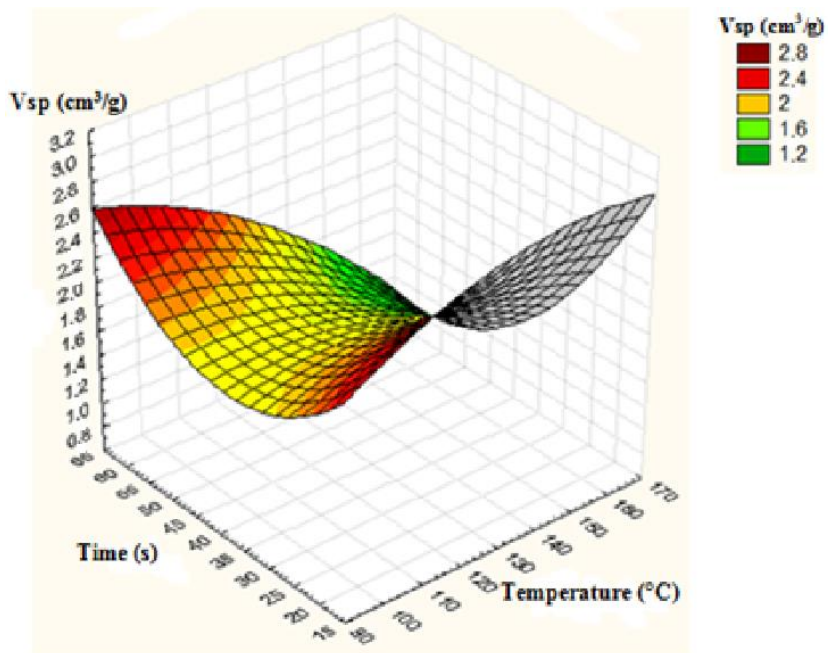


Figure 3. Response surface of specific volume of gluten-free bread of DIC-textured rice-field bean formula according to DIC couple (temperature-time).

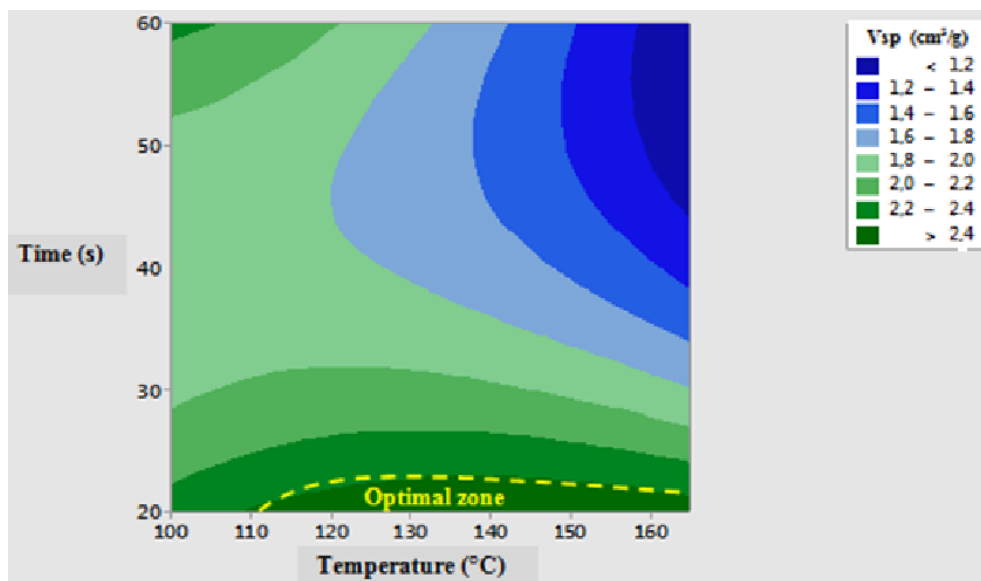


Figure 4. Level curve of specific volume of gluten-free bread of DIC-textured rice-field bean formula according to DIC couple (temperature-time).

that of the wheat bread control ($3.03 \pm 0.10 \text{ cm}^3/\text{g}$), but it was higher than that of the GFB control from untreated formula ($2.45 \pm 0.04 \text{ cm}^3/\text{g}$). The highest values of the Vsp were recorded for a wide range of temperature and steam pressure, coupled with a short treatment time, also for low temperature and steam pressure, and a long treatment time.

Optimization results

The level curve of the Vsp of GFB of DIC-treated rice-field bean formula according to couple (temperature-time) is illustrated in Figure 4. It shows that the highest Vsp values of GFB situated in the optimal zone, in which the Vsp values were higher than $2.4 \text{ cm}^3/\text{g}$, include

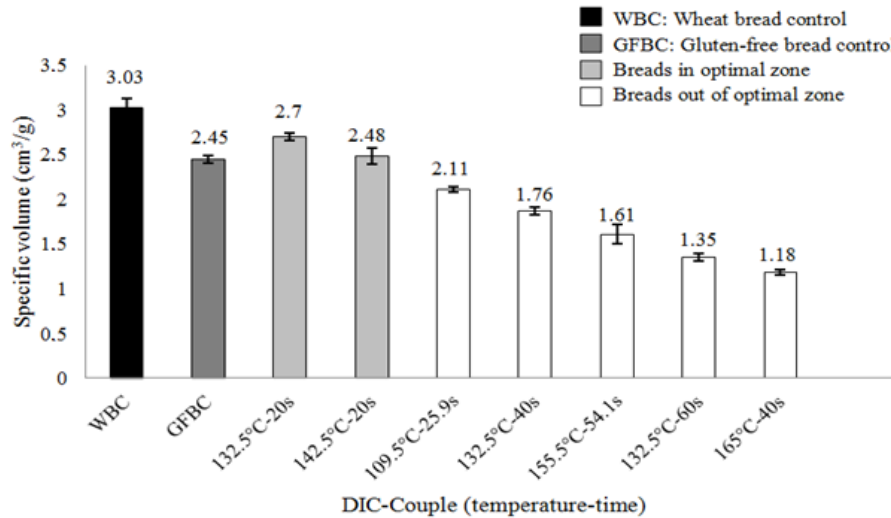


Figure 5. Confirmation points of specific volume of gluten-free breads of DIC-treated rice-field bean formula according to DIC couple (temperature-time).

temperature that ranged from 110 to 165°C, steam pressure from 0.15 to 0.7 MPa, and low treatment time from 20 to 22 s.

Figure 5 represents the confirmation points selected inside and outside the optimal delimited zone of the Vsp of GFB based on DIC-treated rice-field bean formula. All the GFB presented lower Vsp than that of the wheat control bread (3.03 ± 0.10 cm³/g). Obviously, this is due to the absence of structural protein "gluten" in the raw material used in our study (Gallagher et al., 2004). Kawamura-Konishi et al. (2013) demonstrated the inability of rice proteins to form a three-dimensional viscoelastic network similar to that of wheat gluten, which results in low gas retention thus giving bread with low Vsp.

The k-mean classification indicates that the GFB obtained from the treated DIC-rice-field bean formula at 132.5°C (steam pressure of 0.3 MPa) for 20 s giving the highest Vsp of 2.7 ± 0.04 cm³/g would belong in the same class of wheat bread control. In addition, this GFB showed an improvement of Vsp with a gain of 10.20% compared to the GFB control (2.45 ± 0.04 cm³/g). Therefore, it is considered as the optimum GFB of our present study. On the other hand, the GFB of DIC-treated rice-field bean formula at 142.5°C/0.39 MPa for 20 s gave a Vsp of 2.48 ± 0.09 cm³/g. This increase in Vsp of GFB of DIC-treated rice-field bean formula with these conditions compared to the GFB control, would probably be explained by the effect of DIC treatment on the properties of rice flour, which was the basic ingredient used in our GFF. According to Habba and Allaf (1997), Duong et al. (2008), and Pilatowski et al. (2010), the DIC treatment improved and controlled the rice quality compared to the untreated rice sample. Therefore, the Vsp improvement of GFB of DIC-treated rice-field bean formula could be explained by the DIC impacts in terms

of texturing, expanding, and instant cooling. Indeed, the instant pressure-drop towards a vacuum causes an abrupt water autovaporization of the treated sample, inducing a modification of its structure as well as a significant cooling. In addition, Delgado-Rosas et al. (2006) showed that the DIC treatment applied to a maltodextrin powder effectively generates a porous structure with alveoli in the treated sample compared to the untreated one.

According to Prameswari et al. (2018), there is a relationship between the amount of water added during bread making procedure and the ability of flour to absorb water; consequently, the characteristics of bread were affected. Therefore, in our present study, the increase in Vsp of GFB optimum compared to the GFB control could be related to the amount of water added during dough preparation, in which the DIC-treated rice-field bean formula required higher water quantity. Our results are corroborated by those of Setyoprato et al. (2009), who found that the water holding capacity of DIC-treated cassava flour was greater than that of untreated flour. They explain their results by the microstructure change of the treated sample, as the total pore volume and the specific surface. Therefore, the higher amount of water added had a positive effect on the Vsp of GFB based on DIC-treated Rice-Field bean Formula.

On the other hand, the structure of rice flour starch can be affected and modified by the DIC treatment as reported by Onyango (2016), for which this physical treatment (DIC) is one of the methods used to synthesize the physically modified starch. In addition, Onyango (2016) indicated that the damaged starch content of physically treated flours was higher than that of untreated flours, and therefore their water absorption capacity increased, which could be the reason of the Vsp improvement of the GFB optimum.

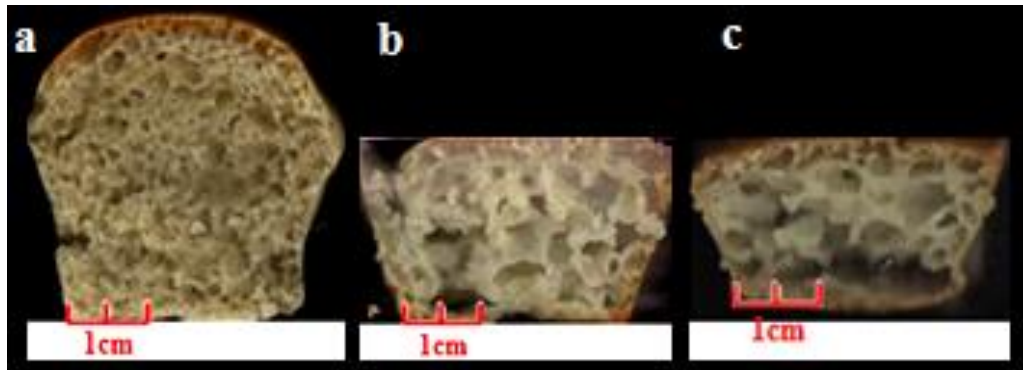


Figure 6. Cross section of bread slices obtained from (a) wheat bread control, (b) gluten-free bread control and (c) gluten-free bread optimum of DIC-treated rice-field bean formula.

Also, it should be noted that the field bean flour incorporated in our GFF has improved the protein content (14.05%) than that of rice flour (6.37%) as previously indicated. According to Verni et al. (2019), field bean flour is characterized by a high protein content (26-39%) of good quality, and its mixture with cereal proteins allows a good balanced amino acid composition. Therefore, we can explain the Vsp improvement of GFB by the positive effect of DIC treatment on protein structure and properties of field bean flour compared to that of untreated formula.

Concerning the points selected outside the optimal delimited zone, the Vsp values recorded are lower than the wheat control and also than the GFB control. They ranged from $1.18 \pm 0.04 \text{ cm}^3/\text{g}$ to $2.11 \pm 0.03 \text{ cm}^3/\text{g}$ for the couple temperature/pressure-time: $165^\circ\text{C}/0.70 \text{ MPa} - 40 \text{ s}$ and $109.5^\circ\text{C}/0.15 \text{ MPa} - 25.9 \text{ s}$, respectively. Therefore, the increase in temperature (increase in steam pressure) and time of DIC treatment causes the decrease of Vsp of GFB of RFF. It is possible to observe that, for the same temperature (same steam pressure), the increase in treatment time causes the decrease of Vsp of GFB. For example, the Vsp was $1.76 \pm 0.04 \text{ cm}^3/\text{g}$ for the conditions $132.5^\circ\text{C}/0.3 \text{ MPa}$ and 40 s, whereas for 60 s it became $1.35 \pm 0.03 \text{ cm}^3/\text{g}$. In addition, the decrease of Vsp is generated by the increase in temperature (increase in steam pressure) for the same treatment time. For instance, for a treatment time of 40 s and a temperature of $132.5^\circ\text{C}/0.3 \text{ MPa}$, the Vsp was $1.76 \pm 0.04 \text{ cm}^3/\text{g}$, whereas at $165^\circ\text{C}/0.7 \text{ MPa}$, it decreases to $1.18 \pm 0.03 \text{ cm}^3/\text{g}$. These decrease in Vsp values could be explained by the intense negative effect of severe DIC conditions treatment (temperature/pressure-time) applied on the GFF of rice and field bean flour.

Structural characteristics of bread crumb

The crumb appearance of the GFB optimum of DIC-treated rice-field bean formula compared to wheat bread


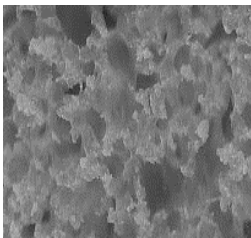
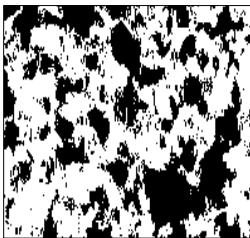

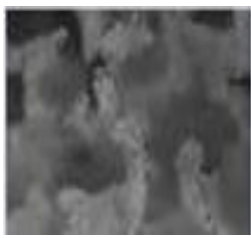
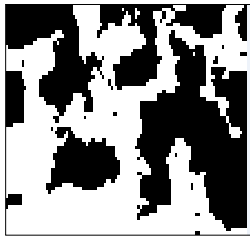

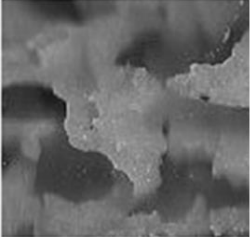

control and GFB control is shown in Figure 6. Our GFB optimum has a well-developed crumb and alveolar structure including a majority of large *alveoli* close to that of GFB control, in opposite, the wheat bread control presents a homogeneous alveolar distribution of small size.

Image analysis results of breads crumb revealed that the *alveoli* total number of GFB optimum of DIC treated rice-field bean formula (28 *alveoli*) was greater than that of GFB control (14 *alveoli*), but it remains inferior than the wheat control bread (110 *alveoli*). Concerning the average size, our breads presented the following values, 0.81, 0.19, and 0.096 for GFB optimum, GFB control and wheat bread control, respectively. In this case, the cells average size of GFB optimum was higher than that of the GFB control and the wheat bread control. Therefore, the DIC treatment of our GFF based on RFF facilitated the air bubbles creation and also favored their development during fermentation. This might be due to the water absorption capacity of treated formula during dough preparation as already mentioned above, as well as the sudden drop of pressure toward vacuum and the rapid autovaporisation of moisture sample during DIC treatment, which could modify the texture and the functional behavior of the material. Indeed, those breads have all a circular *alveoli* shape, with the values close to 1. Consequently, the DIC treatment did not affect the roundness values of the GFB optimum than the GFB control. According to Table 5, the crumb *alveoli* of our GFB optimum and that of GFB control are more open compared to the wheat bread control.

Conclusion

Our study confirmed the considerable contribution of Instant Controlled Pressure Drop (DIC) treatment in the improvement of the technological quality of GFB based on RFF. After performing statistical validation of the obtained model, the effect of DIC operating parameters

Table 5. Crumb representation of the wheat bread control, gluten-free bread control and the gluten-free bread optimum of DIC treated rice-field bean formula.

Type of bread	Real image	Gray level image	Binary image
Wheat bread control			
Gluten-free bread control			
Gluten-free bread optimum of DIC- treated rice-field bean formula			

on the Vsp of GFB was analyzed. The optimization results obtained by the Response Surface Methodology showed the optimal zone including a wide range of temperature between 110 and 165°C corresponding to steam pressures of 0.15 and 0.7 MPa, respectively, and low treatment time ranging from 20 to 22 s. After bread making test, k-means classification indicated that the GFB obtained from DIC treated Rice-Field bean Formula at 132.5°C (steam pressure 0.3MPa) during 20 s is categorized in the same class of wheat bread control, and it is considered as the GFB optimum of DIC treated Rice-Field bean Formula, which gave the highest Vsp of $2.7 \pm 0.04 \text{ cm}^3/\text{g}$ with a gain of 10.20% compared to the GFB control ($2.45 \pm 0.04 \text{ cm}^3/\text{g}$). Based on the results of image analysis of bread crumb, the *alveoli* total number of our GFB optimum of RFF treated with DIC was greater than that of the GFB control. On the other hand, the field bean flour incorporation has improved the nutritional quality of our GFF used in bread making by increasing its protein content. This work carried out in the present study deserved to be completed by in-depth analyzes to have more comprehensive results.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

- American Association of Cereal Chemists (AACC) (1995). Approved methods of the American Association of Cereal Chemists. 9th ed. St. Paul, USA. AACC. Available at: <http://www.sciepub.com/reference/272826>
- American Association of Cereal Chemists (AACC) (2000). Approved methods of the American Association of Cereal Chemists. 10th ed. St. Paul, U.S.A.: AACC. Available at: https://books.google.dz/books/about/Approved_Methods_of_the_American_Associa.html?id=xJwQAQAAMAAJ&redir_esc=y
- Albitar N, Mounir S, Besombes C, Allaf K (2011). Improving the drying of onion using the instant controlled pressure drop technology. *Journal of Drying Technology* 29(9):993-1001.
- Allaf K, Vidal P (1989). Feasibility study of a new process of drying/swelling by instantaneous decompression toward vacuum. Gradient Activity Report, University of Technology of Compiègne UTC N° CR/89/103, industrial SILVA-LAON Partner.
- Allaf T (2013). Application de la Détente Instantanée Contrôlée pour l'Eco-Extraction des Produits Naturels: Intensification & Combinaison. Thèse de doctorat, Université d'Avignon et des pays de Vaucluse, France. Available at: <https://tel.archives-ouvertes.fr/tel-01337322/document>

- Allaf T, Allaf K (2014). Closing Remarks: Instantaneity and Autovaporization. In: Allaf T., Allaf K, editors. Instant Controlled Pressure Drop (D.I.C.) in Food Processing, From Fundamental to Industrial Applications. Food Engineering Series. Springer, NY. pp. 177-178. Available at: <https://www.springerprofessional.de/instant-controlled-pressure-drop-d-i-c-in-food-processing/1894424>
- Benatallah L, Zidoune MN, Michon C (2012). Optimization of HPMC and water addition for a gluten-free formula with rice and field bean based on rheological properties of doughs. *International Review of Chemical Engineering-Rapid Communications* 4(5):474-481.
- Besombes C, Berka-Zougali B, Allaf K (2010). Instant Controlled Pressure Drop extraction of lavender essential oils: Fundamentals and experimental studies. *Journal of Chromatography A* 1217(44):6807-6815.
- Bourekoua H, Benatallah L, Zidoune MN, Rosell CM (2016). Developing gluten-free bakery improves by hydrothermal treatment of rice and corn flours. *LWT-Food Science Technology* 73:342-350.
- Bourekoua H, Różyło R, Benatallah L, Wójtowicz A, Łysiak G, Zidoune MN, Sujak A (2018). Characteristics of gluten-free bread: quality improvement by the addition of starches/hydrocolloids and their combinations using a definitive screening design. *European Food Research and Technology* 244(2):345-354.
- Calle J, Benavent-Gilb Y, Rosell CM (2020). Development of gluten free breads from *Colocasia esculenta* flour blended with hydrocolloids and enzymes. *Food Hydrocolloids* 98:105243.
- Delgado-rosas M, Sobolik V, Allaf K (2006). Texturation de la poudre de maltodextrine par détente instantanée contrôlée. *Sciences des Aliments. International Journal of Food science and Technology* 26(1):103-111.
- Do Nascimento AB, Rataichesk Fiates GM, Teixeira E (2017). We want to be normal! Perceptions of a group of Brazilian consumers with coeliac disease on gluten-free bread buns. *International Journal of Gastronomy and Food Science* 7:27-31.
- Duong TC, Al Haddad M, Rezzoug Z, Laurent Lefevre L, Allaf K (2008). Dehydration by successive pressure drops for drying paddy rice treated by instant controlled pressure drop. *Drying Technology* 26(4):443-451.
- Food and Agriculture Organization (FAO) (1982). Les graines de légumineuses dans l'alimentation humaine. Etude FAO: Alimentation et nutrition 2^{ème} édi, Rome. Italie.
- Gallagher E, Gormley TR, Arendt EK (2004). Recent advances in the formulation of gluten-free cereal based products. *Journal of Food Science and Technology* 15(3-4):143-152.
- Gonzales-Barron U, Butler F (2006). A comparison of seven thresholding techniques with the k-means clustering algorithm for measurement of bread crumb features by digital image analysis. *Journal of Food Engineering* 74(2):268-278.
- Goupy J (2013). Introduction aux plans d'expériences. 5^{ème} édi. Dunod, Paris, France. Available at: <https://excerpts.numilog.com/books/9782100592968.pdf>
- Granato D, de Araújo Calado VM (2014). The use and importance of design of experiments (DOE) in process modelling in food science and technology. In: *Mathematical and Statistical Methods in Food Science and Technology*, 1-18. Wiley, Hoboken.
- Habba A, Allaf K (1997). Instrumental measurement of the cooked rice texture, comparative study between the viscoelastograph and the texturometer, First European Congress Chemical Engineering, Florence, Italy.
- Hager AS, Arendt EK (2013). Influence of hydroxypropylmethylcellulose (HPMC), xanthan gum and their combination on loaf specific volume, crumb hardness and crumb grain characteristics of gluten-free breads based on rice, maize, teff and buckwheat. *Food Hydrocolloids* 32(1):195-203.
- ISO (2009). Method 712: Cereals and cereal products. Determination of moisture content. Available at: <https://www.iso.org/standard/44807.html>
- ISO (2010). Method 2171: Cereals, pulses and by-products. Determination of ash yield by incineration. Available at: <https://standards.iteh.ai/catalog/standards/cen/3df8c44e-68e9-41f6-9c03-8033c660079c/en-iso-2171-2010>
- Kawamura-Konishi Y, Shoda K, Koga H, Honda Y (2013). Improvement in gluten-free rice bread quality by protease treatment. *Journal of Cereal Science* 58(1):45-50.
- Kristiawan M, Sobolik V, Allaf K (2004). Etude comparative d'extraction de l'huile essentielle des fleurs d'ylang-ylang. In: *Proceedings 16èmes rencontres scientifiques et technologiques des industries alimentaires et biologiques - AGORAL*. Montpellier, France. AGORAL -Montpellier, France.
- Lamacchia C, Camarca A, Picascia S, Di Luccia A, Ginafrani C (2014). Cereal based gluten-free food: how to reconcile nutritional and technological properties of wheat proteins with safety for celiac disease patients. *Nutrients* 6(2):575-90.
- Lebwohl B, Sanders DS, Green PHR (2018). Celiac disease. *The Lancet* 391(10115):70-81.
- Lionetti E., Catassi C (2011). New clues in celiac disease epidemiology, pathogenesis, clinical manifestations, and treatment. *International Reviews of Immunology* 30(4):219-231.
- Louka N., Allaf K (2002). New process for texturing partially dehydrated biological products using Controlled Sudden Decompression to the vacuum. Application on potatoes. *Journal of Food Science* 67(8):3033-3038.
- Makharia GK, Verma AK, Amarchand R, Bhatnagar S, Das P, Goswami A, Bhatia V, Ahuja V, Datta Gupta S, Anand K (2011). Prevalence of celiac disease in the northern part of India: a community based study. *Journal of Gastroenterology and Hepatology* 26(5):894-900.
- Masure HG, Fierens E, Delcour JA (2016). Current and forward looking experimental approaches in gluten-free bread making research. *Journal of Cereal Science* 67:92-111.
- Mohammadi M, Azizi MH, Hosseini H, Tirang RN, Mortazavian AM (2015). Development of gluten-free bread using Guar gum and Transglutaminase. *Journal of Industrial and Engineering Chemistry* 21:1398-1402.
- National Council of Food (CNA) (2009). Avis sur le développement des nouvelles technologies dans la fabrication, le conditionnement et la conservation des denrées alimentaires: conséquences, responsabilité des opérateurs et acceptabilité sociale. Avis n° 65:3-19. Available at: <https://cna-alimentation.fr/download/avis-n65-06-2009-avis-sur-le-developpement-de-nouvelles-technologies-dans-la-fabrication-le-conditionnement-et-la-conservation-des-denrees-alimentaires-consequences-responsabilites-des-o/>
- Nguyen TH (2015). Etude expérimentale et modélisation du procédé de séchage des végétaux. Thèse de doctorat, Université de La Rochelle. France. Available at: <https://tel.archives-ouvertes.fr/tel-01297965/document>
- Onyango C (2016). Starch and modified starch in bread making: A review. *African Journal of Food Science* 10(12):344-351.
- O'Shea N, Arendt E, Gallagher E (2014). State of the art of gluten-free research. *Journal of Food Science* 79(6):1067-1076.
- Pilatowski I, Mounir S, Haddad J, Duong TC, Allaf K (2010). The Instant Controlled Pressure Drop Process as a New Post-harvesting Treatment of Paddy Rice: Impacts on Drying Kinetics and End Product Attributes. *Food Bioprocess Technology* 3:901-907.
- Phongthai S, D'Amico S, Schoenlechner R, Rawdkuen S (2016). Comparative study of rice bran protein concentrate and egg albumin on gluten-free bread properties. *Journal of Cereal Science* 72:38-45.
- Prameswari IK, Manuhara GJ, Amanto BS, Atmaka W (2018). Effect of water volume based on water absorption and mixing time on physical properties of tapioca starch-wheat composite bread. *Journal of Physics: Conference Series* 1022:012029.
- Setyopratomo P, Fatmawati A, Allaf K (2009). Texturing by Instant Controlled Pressure Drop (DIC) in the production of cassava Flour: Impact on Dehydration Kinetics, Product physical properties and Microbial Decontamination. *Proceeding of the Word Congress on Engineering and Computer Science* 1:1-6.
- Verni M, Coda R, Rizzello CG (2019). The use of faba bean flour to improve the nutritional and functional features of cereal-based foods: Perspectives and future strategies. In book: *Flour and breads and their fortification in health and disease prevention*. Academic Press 37:465-475.

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