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Effect of postharvest management on the microbial quality of potato (*Solanum tuberosum* L.) tubers

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Abstract. This experiment was conducted to appraise the role of the curing phytohormone treatment and storage method in the postharvest microbial decay of ware potatoes during storage. The study was designed as a Split-Split-Plot Design (SSPD) in which the curing period was placed in the main plot, and the storage method and the phytohormone were put in subplot and sub-sub-plot respectively. Each treatment was replicated three times to form a $4 \times 3 \times 3$ factorial experiment. Each treatment consisted of 20 potato tubers out of which decayed samples were counted for the calculation of percentage rot loss and identification of the causal agent, which was done fortnightly until the end of the storage period of 12 weeks. Temperature, relative humidity, and wind velocity were monitored weekly. All data collected were subjected to analysis of variance (ANOVA) using the GenStat Discovery Edition statistical software package. Means that exhibited significant difference were separated using Duncan's Multiple Range Test (DMTR) at $p = 0.05$ level of significance. The results of the analysis showed that the phytohormone levels had highly significant influence ($p \leq 0.01$) on percentage rot loss. Although there was a significant interaction between the curing periods and phytohormone concentration, storage conditions ($p \leq 0.05$) were observed throughout the storage period of 2–8 weeks. *Fusarium solani*, *Rhizopus oryzae*, and *Aspergillus niger* were implicated in the rotting of potato tubers.

Keywords and phrases: storage rot loss, potato, *Aspergillus niger*, *Fusarium solani*, *Rhizopus oryzae*

1. Introduction

The short production cycle of potatoes (*Solanum tuberosum* L.) makes it possible to supply food faster than most crops, such as cereals or legumes, during emergencies and famine (*Mustefa et al.*, 2017; *Devaux et al.*, 2020). Furthermore, they yield more calories per hectare and require a relatively lower quantity of water when compared to other crops such as wheat or rice (*Sonnewald & Sonnewald*, 2014; *Thapa & Thapa*, 2019; *Burgos et al.*, 2020). Potato tubers are an excellent staple food due to their high nutritional value, which, in addition to their relatively easy cultivation, makes potato increasingly important as a critical crop to feed an ever-increasing world population (*Lisinska et al.*, 2008; *Pandey et al.*, 2017). According to *Burgos et al.* (2020) and *Devaux et al.* (2020), potato is a very important part of world food security, which could help to alleviate poverty, drive economic development, and sustain rural livelihood.

Quality losses caused by biotic factors, such as microbes, have been estimated at 6.1 billion euros with a serious impact on food security (*Adolf et al.*, 2020). Postharvest loss of horticultural crops is about 40-50% (*Teutsch & Kitinoja*, 2019), which is mostly caused by poor postharvest handling and microbial decay. Globally, postharvest losses have recently become a serious source of concern, which made the United Nations to declare Food Loss and Waste (FLW) as one of the Sustainable Development Goals (SDGs) to reduce FLW by 50% by 2030 so as to feed the ever-growing population of the world (*Teutsch & Kitinoja*, 2019). In addition to that, resource-constrained nations are facing challenges in power availability, accessibility, and affordability to embark on cold chain technology.

Other researchers, such as *Lulai et al.* (2008) or *Olsen & Miller* (n. y.), have previously observed that the curing period assisted significantly the control of microbial decay during storage through wound healing or suberization. *Wang et al.* (2020a) stated that curing healed broken potato skin thereby putting a stop to microbial infection, thus combatting microbial attack during storage. Likewise, *Holcroft* (2018), *Jiru & Usmane* (2021) reported that curing helps in minimizing rotting during storage.

Alamar et al. (2017) opined that poor storage conditions of potato tubers can cause significant losses during their postharvest life. Root and tuber crops are more susceptible to microbial deterioration under certain storage conditions, as observed by *Sugri et al.* (2017), *Teme et al.* (2019), *Wang et al.* (2020b), and *Jiru & Usmane* (2021), who reported that storage conditions have a direct impact on the rotting of potato tubers in storage.

Postharvest exogenous application of ABA on micro tubers was found to aid in the control of postharvest disease of potato tubers during storage, as documented by *Adolf et al.* (2020). Similar findings were reported by *Alamar et al.* (2017),

Chen et al. (2020), *Wang et al.* (2020b), and *Haider et al.* (2021) – they all noted that ABA plays a significant role in the control of microbial spoilage of potatoes through dormancy induction and maintenance.

There is little information about the influence of phytohormone, storage method, and curing on the microbial loss of potatoes during storage. The minimization of these losses is the aim of this research, which seeks a means of improving potato storage longevity under ambient conditions that would control microbial rotting through the use of curing, abscisic acid (ABA), and storage methods.

2. Materials and methods

The materials and equipment for this research include:

1. Fresh, fully matured dry-season potato tubers (Marabel cv. seeds) were obtained from the National Root Crops Research Institute (Jos, Nigeria), which were harvested early in the morning in Kwaja village (Mubi, Nigeria).
2. Abscisic acid (ABA) with a purity of 95% was purchased from Zhengzhon Panpan Chemical Co. Ltd, China.
3. Thermo-hygrometer HTC-2.
4. Automatic Digital Weather Station, Vantage Pro2 model (Davis Instruments, Hayward, CA, USA).

2.1 Curing periods

The curing of potato tubers involves keeping the tubers underneath a tarpaulin cover under high temperature (35°C) and relative humidity (80%) conditions, in a dry, shaded place without ventilation. The tubers were subjected to the following curing periods:

a. No curing period [control] (P_0)
b. Three days curing period (P_1)
c. Five days curing period (P_2)
d. Seven days curing period (P_3)

2.2 Levels of phytohormone (abscisic acid)

The following amounts of abscisic acid were applied to the experimental samples of the potato tubers:

a. Without treatment [control] c = 0 ppm (A_0)
b. Treatment with concentration of c = 2 ppm (A_1)
c. Treatment with concentration of c = 4 ppm (A_2)

2.3 Storage conditions

The following storage methods were employed for the research:
a. C_0: Floor storage on bare concrete floor (control).
b. C_1: Heap storage between layers of paddy straw.
c. C_2: Shaded pit storage (50 × 50 × 70 cm) with alternate layers of paddy straw under shade.

2.4 Experimental design

The experiment was prepared in a Split-Split-Plot Design (SSPD) with curing applied to the main plot while storage conditions and ABA levels apportioned between the sub-plot and the sub-sub-plot. Treatment was replicated three times (4 × 3 × 3 factorial experiment), and temperature, relative humidity, and wind speed of the storage environment were monitored using both a digital thermo-hygrometer and a digital automatic weather station during the experiment. Each treatment consisted of 20 potato tubers, out of which three were labelled and earmarked for data collection.

2.5 Sampling methods and data collection

Purposeful sampling technique was used in the selection of the decayed samples on which data were generated. Data were taken fortnightly on the percentage of rotting parameters, whilst samples for microbial analysis were collected at the end of the storage period. The following methods were used to collect the data:

a. Determination of storage rot loss percentage (% SRL):

Potato tubers with a visibly decayed surface area were considered as rotted according to *Ezeocha & Ironkwe* (2017). Further, the storage rot losses of tubers (% SRL) was calculated by counting the rotted tubers out of the total tubers in each treatment after each sampling period and expressed in percentage. Storage rot loss (% SRL) was calculated according to the following formula (1), as recommended by *Brar & Rana* (2016) and *Abubakar et al.* (2019):

$$\% \, SRL = (1 - \frac{N_r}{N_t}) \times 100, \tag{1}$$

where: N_r = number of remaining potato tubers, N_t = total number of potato tubers.

b. Microbiological analysis – characterization and identification of isolates:

The samples were subjected to the method of food examination involving the detection of the number and activity of viable microorganisms to ascertain the presence of decay causal agent. The agent was isolated and identified. Rotten

samples were collected from the potato tubers at the end of every sampling period and transferred into Petri dishes containing appropriate solid substrate (agar) media and cultured so that they can be eventually identified visually. The dishes were incubated for 48 hours at 37°C. The interest here is to determine the existence of viable microorganisms (bacteria, viruses, and microfungi) without counting them necessarily; thus only to isolate and identify them as recommended by *Onwuka* (2005) and *Ijah et al.* (2014).

The collected data were subjected to analysis of variance (ANOVA) using the generalized linear model. Means that showed significant differences were separated using less significant differences (LSD) at the confidence level of 95%.

3. Results and discussion

3.1 Effect of experimental parameters (curing period (CRP), storage conditions (STC), and abscisic acid concentration (ABA level)) on potato storage rot loss (SRL) during different storage durations

3.1.1 Effect of experimental parameters on storage rot loss (SRL) during different storage durations

Throughout the sampling periods, no significant effect ($p > 0.05$) was found on potato tubers subjected to varying curing periods with regard to storage rot loss (*Table 1*). This result is in disagreement with *Mehrotra & Aggarwal* (2004), who earlier reported that curing periods affected potato tuber decay and water loss. A similar tendency could be observed concerning the impact of storage conditions on storagse rot loss: there was no significant effect ($p > 0.05$) at all sampling periods, as evident in *Table 1*. This finding was again contrary to *Mehrotra & Aggarwal* (2004) and *Sugri et al.* (2017), who claimed that storage conditions influence the decay of potato tubers during storage and transit.

A highly significant influence ($p \leq 0.001$) of the ABA rate on percentage rot loss was observed only at 8 weeks of storage duration (*Table 1*). The highest rot loss was reported on samples treated with 2 ppm (2.50%), while the lowest rot loss was reported for potato tubers without ABA treatment and also at 4 ppm (0 and 1.11% respectively), but both treatments are statistically alike. This outcome is in accordance with *Mehrotra* & *Aggarwal* (2004), *Haider et al.* (2021), and *Wang et al.* (2020), who confirmed that some plant growth regulators help to control *Fusarium*, *Rhizopus*, and *Aspergillus* rotting during the storage of fruits and vegetables.

Accordingly, potato tubers cured in all curing periods and all ABA levels and stored on the floor experienced almost no rotting throughout the storage periods. Specifically,

treated potato tubers with 4 ppm ABA, stored for three days in both heap and shaded pit storage had no rotten tubers at the end of the study, as shown in *Table 1*.

Table 1. Statistical results of the effect of parameters on storage rot loss

Storage period (weeks)	2	4	6	8	10	12
Treatment						
CRP (days)						
0	60.8a	1.11a	1.48a	1.48a	0.00a	0.00a
3	53.2a	1.11a	0.37a	1.85a	0.00a	0.00a
5	33.3a	0.74a	0.00a	1.11a	0.00a	0.00a
7	48.4a	0.37a	1.48a	0.37a	0.00a	0.00a
P of F	0.285	0.815	0.198	0.606	-	-
S.E. (±)	12.96	0.896	0.741	1.101	0.00	0.00
STC						
Floor	45.9a	0.83a	0.83a	1.11a	0.00a	0.00a
Heap	48.9a	1.11a	0.83a	1.39a	0.00a	0.00a
Pit	51.9a	0.56a	0.83a	1.11a	0.00a	0.00a
P of F	0.895	0.693	1.000	0.848	-	-
S.E. (±)	12.69	0.661	0.786	0.556	0.00	0.00
ABA (ppm)						
0	39.2a	0.83a	0.83a	0.00b	0.00a	0.00a
2	57.8a	1.11a	0.83a	2.50a	0.00a	0.00a
4	51.9a	0.56a	0.83a	1.11b	0.00a	0.00a
P of F	0.261	0.704	1.000	**0.003**	-	-
S.E. (±)	11.22	0.661	0.600	0.680	0.00	0.00
Interactions						
CRP × STC	NS	NS	NS	NS	-	-
CRP × ABA	*	NS	NS	*	-	-
STC × ABA	*	NS	*	*	-	-
CRP × STC × ABA	NS	NS	NS	NS	-	-

Notes: Means with the same letter are not significantly different; * significant, ** highly significant.

This finding could be attributable to environmental factors such as low temperature, low relative humidity, and high air speed to which the samples were exposed during storage. This condition inhibited microbial development,

as also supported by *Mehrotra* & *Aggarwal* (2004) and *Wang et al.* (2020), who earlier observed that low temperature, relative humidity, and good ventilation influence the development of *Fusarium* storage rot of potato tuber. They further opined that this infection probably occurred already in the field, where the soil is infested. The result of this work is also in alignment with *Mani et al.* (2014) and *Wang et al.* (2020), who confirmed the role of curing and storage environment in the rotting of potato during storage. It is also in line with *Sugri et al.* (2017), who reported that postharvest management affects the decaying of tubers by fungi such as *Aspergillus niger*, *Fusarium oxysporum*, or *Rhizopus stolonifer*.

3.1.2 Influence of the interaction between curing period and ABA level on storage rot loss at two weeks of storage

The mean interaction effect between curing period and ABA level on the percentage storage rot loss of potato tubers during storage was significant ($p \leq 0.05$), as shown in *Figure 1*. The lack of ABA treatment and a three days curing period yielded the highest rot loss of 54.6%, while the lowest rot loss occurred after a seven days curing period (15.5%); however, after adding 2 ppm of ABA, potato tubers cured for three days recorded the greatest loss of 90.8%, whereas tubers cured for five days recorded the smallest loss of 32.9%. Following a successive increase of the ABA level to 4 ppm, both uncured and five days cured tubers presented the highest loss (74.9%), and at the same time tubers cured for three days displayed the smallest loss (14.3%). This could be due to both abiotic and biotic factors such as pathogens and low humidity. This finding is in concordance with *Sugri et al.* (2017) and *Chen et al.* (2020), who observed that curing and ABA influence rotting.

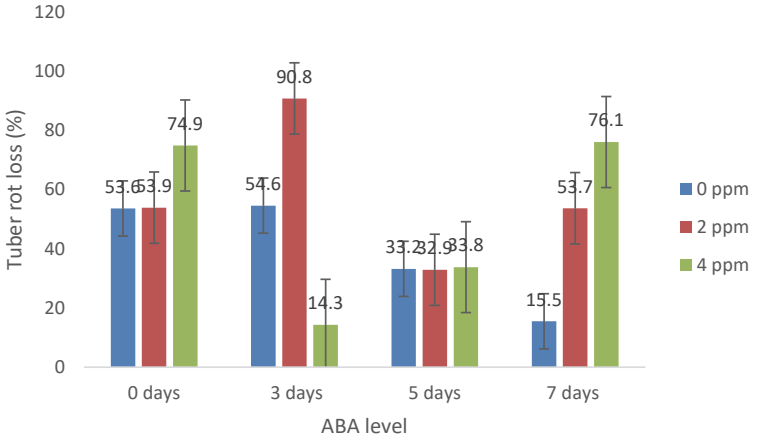

Figure 1. Interaction between curing period and ABA level at two weeks of storage

3.1.3 Interaction between storage conditions and ABA level at two weeks of storage

Storage conditions and ABA levels exhibited a significant ($p \leq 0.05$) interaction at the storage time of 2 weeks, as presented in *Figure 2*. In addition, potatoes stored on the floor had the highest value of 63.6% and the lowest value of 16.7% for shaded pit storage without ABA treatment, and when potatoes were treated with 2 ppm ABA level, potatoes stored in a shaded pit recorded the highest value of 71.7%, and the lowest value was obtained for floor storage, with 37.6%. With a supplementary increase of ABA from 2 ppm to 4 ppm, tubers stored in heap continued to yield the highest rot loss of 67.5%, while the lowest rot loss of 36.6% was observed on potatoes stored on floor. This could be due to the effect of the interplay between storage conditions and ABA on the percentage rot loss, which is in line with *Mani et al.* (2014) and *Wang et al.* (2020b), who reported that storage conditions and ABA treatment affect rotting.

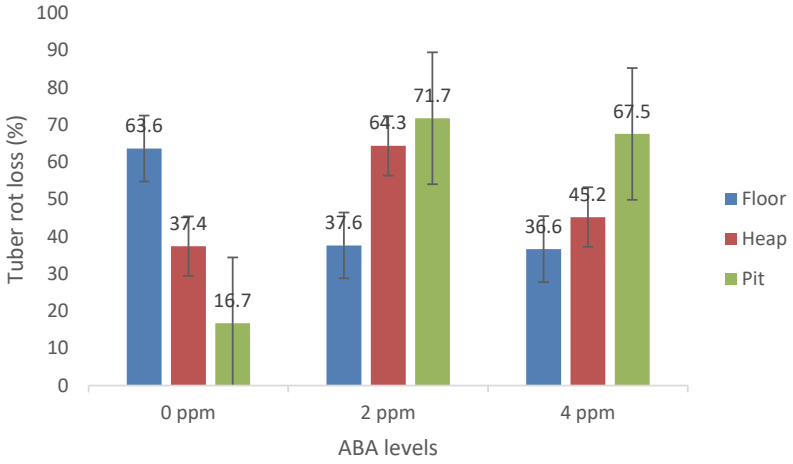

Figure 2. Interaction between storage conditions and ABA level at two weeks of storage

3.1.4 Interaction between storage conditions and ABA levels at six weeks of storage

Storage conditions and ABA levels demonstrated a significant ($p \leq 0.05$) interaction at the storage time of 6 weeks, as shown in *Figure 3*. Similarly, potatoes stored on the floor had the greatest rot loss of 1.67% and the least rot loss of 0.00% on shaded pit storage without ABA treatment (0 ppm), and when ABA was increased to 2 ppm, potatoes stored in shaded pit yielded the greatest value of

2.50%, and the least loss of 0.00% was on potatoes stored on floor and in heap. Following an additional rise of the ABA level to 4 ppm, potatoes stored in heap continually showed the greatest rot loss of 1.67%, and the least of 0.00 % (no rot loss) was spotted on potatoes stored in a shaded pit. This may be attributable to the double effect of storage conditions and ABA on rot loss, which is in tandem with *Mani et al.* (2014) and *Wang et al.* (2020), who reported that storage conditions and ABA impacted microbial spoilage.

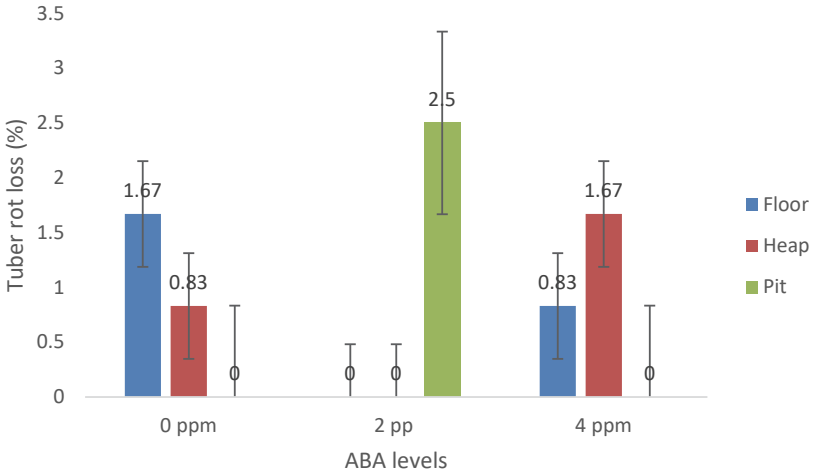

Figure 3. Interaction between storage conditions and ABA level at six weeks of storage

3.1.5 Interaction between curing period and ABA level at eight weeks of storage

The mean interaction effect between curing period and ABA level on the percentage storage rot loss of potato tubers during storage was significant ($p \leq 0.05$), as shown in *Figure 4*. When ABA was not applied, all the curing periods under consideration recorded no rot loss (0.00%), but after adding 2 ppm of ABA, potato tubers cured for three days recorded the highest loss of 5.56%, whilst tubers cured for both five and seven days recorded each the lowest loss of 1.11%. Upon a successive increase of the ABA level to 4 ppm, tubers cured for both zero and five days gave the highest loss of 2.22% each, and at the same time tubers cured for both three and seven days recorded the lowest loss value of 0.00%. This may be owing to the combined influence of curing and ABA on rot loss. This result concurs with *Sugri et al.* (2017) and *Chen et al.* (2020), who observed that curing and plant growth regulators inhibits rotting.

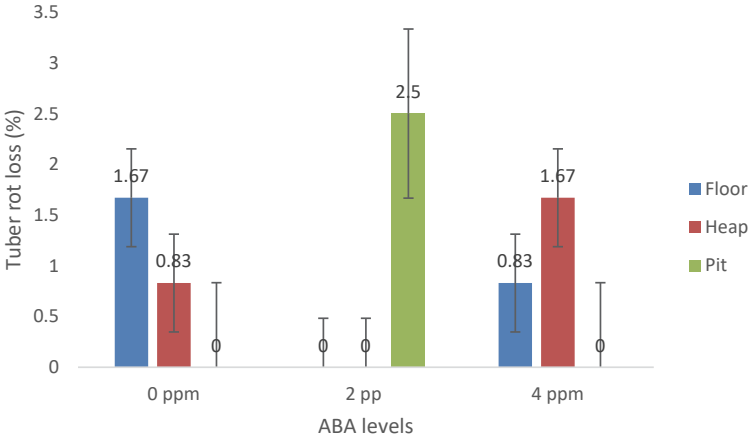

Figure 4. Interaction between curing periods and ABA level at eight weeks of storage

3.1.6 Interaction between storage conditions and ABA level at eight weeks of storage

There was a significant ($p \leq 0.05$) interaction effect of storage conditions and ABA rates at a storage time of 8 weeks on rot loss, as presented in *Figure 5*. In the absence of ABA treatment, no rot loss (0.00%) was recorded regardless of storage conditions; however, adding 2 ppm of ABA led to the highest rot loss (4.17%) of potatoes stored in heap and the smallest (0.83%) loss of those stored in a shaded pit. An additional increase of ABA to 4 ppm triggered potatoes stored in shaded pit to take the lead (2.25% each) in terms of rot loss, while those stored in heap yielded no rot loss (0.00% each). This outcome attests to the impact of the interaction between storage conditions and ABA on the percentage of rot, loss which is concurrent with *Mani et al.* (2014) and *Wang et al.* (2020b), who confirmed that curing and phytohormones play a significant role in the microbial deterioration of potatoes.

Table 2 presented the rotting level of potato tubers subjected to varying curing periods, storage conditions, and ABA levels during 12 weeks of storage under ambient conditions. Bacteria and viruses were not recorded as infectious causal agents for potato rotting during this research. However, fungal attack was noticed as the sole agent responsible for tuber decay on all of the rotten potato tubers.

Despite that, the majority of the samples under study did not record deterioration due to microbial infection. Nonetheless, potato tubers cured for seven days suffered no tuber decay; this was followed by zero days curing with 6 rotten tubers, while the curing periods of three and five days had the highest decay of 9 and 10 tubers respectively. The floor storage method exhibited a minimum deterioration of only

1 tuber followed by shaded pit storage, which had 6 rotten tubers, whereas heap storage conditions recorded the highest number of rotten tubers (18). In the case of ABA treatment, no ABA treatment with 2 ppm ABA concentration had the lowest deterioration (5 tubers), whilst the highest deterioration (13 tubers) was observed on tubers treated with 4 ppm ABA.

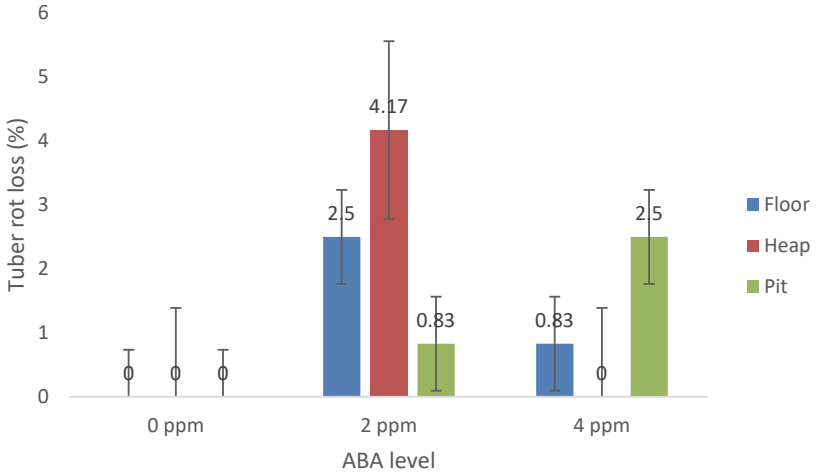

Figure 5. Interaction between storage conditions and ABA level at eight weeks of storage

Three days curing period with heap storage and treated with 2 ppm of ABA level recorded the highest (5 tubers) rotting percentage due to fungal attack at all the sampling periods except at 2 weeks duration of storage time. Next are tubers cured for three days, sprayed with 4 ppm ABA, and stored in heap. Those cured for five days, treated with 4 ppm of ABA, and stored in a shaded pit with 4 tubers each showed infection from 6 to 12 weeks storage time, which was the end of the storage period.

Similarly, samples cured for five days, stored in heap, and untreated with ABA and those cured for five days, stored in heap, and treated with 4 ppm of ABA had 3 tubers decayed in the second half of the storage period (8, 10, and 12 weeks of storage time). Further, uncured potato tubers stored in heap with 2 ppm of ABA experienced fungal spoilage on 2 tubers only in the last two weeks of storage (10 and 12 weeks duration of storage time). Uncured samples with 4 ppm ABA that were stored in a shaded pit all suffered fungal infection on a tuber each in the 12 weeks duration of storage time.

Table 2. The effect of parameters on the rotting level of potato tubers during storage

Treatments								
CRP	STC	ABA	Storage period (weeks)					
(days)		(ppm)	2	4	6	8	10	12
0	Heap	0	-	-	-	-	-	+
3	Heap	0	-	-	-	-	-	-
5	Heap	0	-	-	-	+	+	+
7	Heap	0	-	-	-	-	-	-
0	Heap	2	-	-	-	-	+	+
3	Heap	2	-	+	+	+	+	+
5	Heap	2	-	-	-	-	-	-
7	Heap	2	-	-	-	-	-	-
0	Heap	4	-	-	-	-	-	+
3	Heap	4	-	-	+	+	+	+
5	Heap	4	-	-	-	+	+	+
7	Heap	4	-	-	-	-	-	-
0	Floor	0	-	-	-	-	-	-
3	Floor	0	-	-	-	-	-	-
5	Floor	0	-	-	-	-	-	-
7	Floor	0	-	-	-	-	-	-
0	Floor	2	-	-	-	-	-	+
3	Floor	2	-	-	-	-	-	-
5	Floor	2	-	-	-	-	-	-
7	Floor	2	-	-	-	-	-	-
0	Floor	4	-	-	-	-	-	-
3	Floor	4	-	-	-	-	-	-
5	Floor	4	-	-	-	-	-	-
7	Floor	4	-	-	-	-	-	-
0	Pit	0	-	-	-	-	-	-
3	Pit	0	-	-	-	-	-	-
5	Pit	0	-	-	-	-	-	+

Treatments								
CRP	STC	ABA	Storage period (weeks)					
(days)		(ppm)	2	4	6	8	10	12
7	Pit	0	-	-	-	-	-	-
0	Pit	2	-	-	-	-	-	-
3	Pit	2	-	-	-	-	-	-
5	Pit	2	-	-	-	-	-	-
7	Pit	2	-	-	-	-	-	-
0	Pit	4	-	-	-	-	-	+
3	Pit	4	-	-	-	-	-	-
5	Pit	4	-	-	+	+	+	+
7	Pit	4	-	-	-	-	-	-

In terms of curing period, the five days curing period resulted in the highest decay (11 tubers) across all storage conditions and ABA levels, which was followed by three days curing period with 9 decayed tubers, and seven days curing period recorded no decayed tubers under all storage conditions and across all ABA levels. Similarly, in the case of storage conditions, heap storage led to the highest number of rotten tubers, with 19 tubers affected, followed by pit storage with 6 rotted tubers, while floor storage had the least with only one rotten tuber in all the curing periods and ABA levels.

Tubers treated with 4 ppm ABA experienced the highest rotting (13 tubers). This could be the ABA threshold level that influences rotting, followed by those treated with 2 ppm (8 tubers), and the tubers least infected by fungi under all curing periods and storage conditions were the control tubers (5).

The characterization and identification of fungal isolates were done based on macroscopic and microscopic examination. Three species of fungi were isolated and identified, namely: *Fusarium solani*, *Rhizopus oryzae*, and *Aspergillus niger*. This outcome is similar to *Mehrotra* & *Aggarwal* (2004) and *Ijah et al.* (2014), who identified *Fusarium*, *Rhizopus*, and *Aspergillus* among the major causal agents responsible for causing rot in potato tubers and its products. Similarly, *Sugri et al.* (2017) pointed out that sweet potato was also substantially ravaged by rot-causing pathogens, such as *Aspergillus* and *Fusarium*, during storage. Earlier it was observed that *Fusarium* storage rot of potato tubers occurs in the field, where the high level of inoculum is heavily present in the soil. Similarly, the fungus *Rhizopus oryzae* could be from the rice straw that was used in both heap and shaded pit storage, which may be infested with the pathogen.

4. Conclusions

This study concluded that ABA level at 4 ppm recorded the best rot loss (1.11%), while both curing period and storage conditions with ABA level gave the best results, the highest rot loss at a storage duration time of 8 weeks. The study also identified and characterized *Fusarium solani*, *Rhizopus oryzae*, and *Aspergillus niger* as the major causal agent of the rotting of potato tubers in the study area. Furthermore, potato tubers cured for three days with 4 ppm ABA level and stored in both heap and shaded pit storage had no rotten tubers during the study.

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Classification of different types of flours available in the Romanian market based on the nutrition content

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Abstract. Flour is one of the most widely used products typically derived from wheat, corn, and rye and is classified based on its nutrition content. The present study aimed to identify and classify the different types of flour (wheat, rye, maize) commercially available in Romania. The market research covered eight types of flour available on the Romanian market: 1. Wheat Flour 000 (N-19), 2. Wheat Flour 550 (N-11), 3. Wheat Flour 650 (N-13), 4. Whole-Wheat Flour (N-15), 5. Durum Wheat Flour (N-8), 6. Spelt Wheat Flour (N-8), 7. Rye Flour (N-15), and 8. Maize Flour (N-23). The classification was carried out by analysing the most important parameters: energy, protein, fat, saturated fatty acid, carbohydrate, sugar, and dietary fibre content, using different statistical methods: descriptive statistics, box plot, hierarchical cluster, and surface radar analysis. The results revealed that white wheat flour represented more than 50% of the analysed samples, and significant differences were found between the tested types of flour. In addition, white wheat flour is characterized with lower dietary fibre, fat and protein content, but it has higher energy content and carbohydrate content. In contrast, whole meal, durum, spelt, and rye flours are characterized by lower carbohydrate

and higher fibre and fat content. The hierarchical cluster analysis showed that, on the basis of nutritional similarities and differences, the flours studied in Romania can be grouped into three distinct clusters.

Keywords and phrases: wheat, flour, carbohydrate, dietary fibre, nutrition value

1. Introduction

The different types of flour are produced primarily by using grain milling technologies, which have undergone major changes and developments from the beginning to the present. The milling of cereals is as old as mankind, with the earliest archaeological findings dating back to 6000 BC, and the flours produced played a fundamental role in human nutrition, which are still widely used in the world and are excellent sources of protein, complex carbohydrates, fibre, or even vitamins. According to different statistical reports, the total wheat consumption worldwide has increased from 742.82 to 759.54 million tonnes over the last four years (*Statista 1*, 2022). The top seven cereals grown in the world are maize, wheat, rice, barley, sorghum, oat, and rye, in the proportions of 1.125, 775.8, 505, 159.74, 62.05, 25.53, 14.3 million tonnes respectively (*Statista 2*, 2021).

The market offer of the different types of flour is much diversified, depending on the part of the grain that remains after milling. Within the European Union, thanks to the EU's single market, flour produced in any EU country can be bought and compared on the market of a given country. The grain kernel is structurally made up of three major components: the husk, the kernel core, and the germ (*Olugbire et al.*, 2021; *Zafar et al.*, 2020).

White flour generally contains only the endosperm part of the grain, which is high in carbohydrates, protein and has a small amount of oil. If all the husk content is milled into the flour during the milling process, wholemeal flour is obtained, which is darker in colour and has a higher fibre content (*Goesaert et al.*, 2005; *Zhang*, 2020).

Furthermore, the types of flour that contain the germ (reproductive epicentre) are rich in vitamins, minerals, oil, and fibre as well. While gluten is a unique protein found in wheat flour and products, it provides elasticity to the raw dough and helps to retain the gases produced during dough maturation (*Gómez et al.*, 2020).

According to the literature, there are different types of flour: all-purpose flour, unbleached flour, bread flour, cake flour, pastry flour, self-rising flour, and whole-wheat flour. The protein content, especially the gluten quality and quantity, is responsible for the rheological properties of raw pasta (*Araujo et al.*, 2008; *Ciudad-Mulero et al.*, 2021).

Therefore, flours with higher protein content provide a higher gluten ratio and a stronger dough for products such as bread, while low-protein flours are suitable for cakes and biscuits (*Hughes & Vaiciurgis*, 2020).

In addition to wheat flour, the baking industry also uses rye flour as supplementary flour, and corn flour has a wide range of uses, such as cornflakes and polenta, which is the national dish of the Romanian population.

The fat content of flour is used as a technological indicator, hence increased fat content can increase the volume of the bread as well by helping in the incorporation and retention of air during dough mixing (*Pareyt et al.*, 2011). It also imparts fat sensitivity, moisture, spread ability, flavour, colour, and anti-caking properties to bakery products (*Dewettinck et al.*, 2008).

The main objective of this research was to collect and compare the nutritional values of different types of flour commercially available in Romania in order to provide a comprehensive picture for consumers and users regarding the nutrient content.

2. Materials and methods

In this study, a market control was conducted in all existing supermarkets in Miercurea Ciuc (Romania) in August 2021 – a city of approx. 34,500 inhabitants (based on the 2021 census), and online shops available in Romania were also included. A total of 113 different flour nutritional values were collected from product labels, including: energy, fat, saturated fatty acids, carbohydrate, sugar, fibre, and protein content, per 100 g of flour. The origin of the examined flour products covers not only the Romanian production but also the products of several EU Member States (Supplementary Tables 1–8). Eight flour categories were analysed separately: 1. Wheat flour 000 (N-19), 2. Wheat flour 550 (N-11), 3. Wheat flour 650 (N-13), 4. Whole-grain wheat flour (N-15), 5. Durum wheat flour (N-8), 6. Spelt wheat flour (N-8), 7. Rye flour (N-15), 8. Corn flour (N-23). The collected data were analysed by using different statistical methods. For the 95% confidence interval calculation, the following equation was used (Equation 1):

$$\bar{x} - t * \frac{s}{\sqrt{n}} \leq \mu \leq \bar{x} + t * \frac{s}{\sqrt{n}}, \tag{1}$$

where $\bar{x}$ = the average, t = Student t, s = standard deviation, and n = sample size.

In addition, descriptive statistics (Minimum, 25th percentile, Median, Average, 75th percentile, Maximum) were used to find patterns among the data and characterize the different flours. The data distribution was determined by using the widely adopted box plot method, and surface radar charts were created using Microsoft Excel 2016 in order to compare the characteristics of the samples.

Before performing the cluster analysis, the data were verified, and the average values of the energetic value as well as of the fat, fatty acid, carbohydrate, sugar, and protein content were used. The applied parameters were centroid linkage

and Euclidian distance using IBM SPSS Statistics 22 software. The hierarchical relationships of the flour samples were visualized as a dendrogram. Centroid linking is a commonly used method that attempts to find a user-specified number (K) of clusters represented by their centroids. The hierarchical cluster analysis examines the point at which pairs that are related earlier are more similar than those that are related later in order to clarify common sources. To perform the hypothesis tests, the f-test and the two-sample t-test of the Microsoft Excel Data Analysis program package were carried out.

According to the GD 106/2002, the below wheat flour category specifications were established (*Table 1*). Based on the WHO recommendations, the maximum permitted moisture content is 15.5% (*Food and Agriculture Organization of the United Nations and World Health Organization*, 2021).

Table 1. The wheat flour classifications based on the standards (Ministry of Health, 2009)

Characteristics	White flour				Semi-white	Black flour	Black dietary
	Type 480	Superior type 000	Type 550	Type 650			
Humidity (max. %)	14.5						
Acidity degree (max.)	2.2			2.8	3.2	5	5
Ash content reported to the dry substance (max. %)	0.48		0.55	0.65	0.66–0.90	0.91–1.4	1.41–2.2
Insoluble ash content in HCl 10 (max. %)	0.2						

3. Results

3.1 Statistical analysis of the studied flours

The nutritional data of 113 flour products were collected and classified during the experiment. The nutritional values of the studied flour types are presented in *Table 2*, where the means and 95% confidence intervals (Mean ± 95% CI) are given. The results revealed that in terms of energy content the rye flour has the lowest value with 1,376 kJ/100 g, while the highest one was in the case of the

durum wheat flour: 1,487 kJ/100 g. In terms of fat and saturated fatty acid content, the lowest values were identified for spelt wheat, with 2.35 g/100 g and 0.52 g/100 g, respectively, and this category of flour has been characterized by the lowest carbohydrate percentage, 63.42 g/100 g, and the highest sugar and protein content, 2.72 g/100 g and 13.79 g/100 g respectively. From a dietetic point of view, this type of flour has the most favourable composition (*Wieser & Kieffer*, 2001). Regarding the dietary fibre content, the highest value (higher than 7 g/100 g) was found in the case of rye, whole-grain flour, and spelt flour.

Table 2. The nutrient composition of the main flour categories per 100 g

	(mean ± conf. int., α = 0.05)	Energetic value, kJ	Energetic value, kcal	Fat, g	Fat of which saturated fatty acids, g	Carbohydrates, g	Carbohydrates of which sugars, g	Dietary fibre, g	Protein, g
Wheat flour 000	***Avrg.***	***1,467***	***346***	***0.96***	***0.18***	***72.8***	***1.02***	***1.69***	***10.7***
	CI	*±25.61*	*±6.12*	*±0.07*	*±0.03*	*±1.55*	*±0.35*	*±0.55*	*±0.3*
Wheat flour 550	***Avrg.***	***1,448***	***345***	***1.07***	***0.3***	***70.7***	***1.73***	***2.68***	***10.7***
	CI	*±13.22*	*±6.5*	*±0.16*	*±0.09*	*±0.84*	*±0.96*	*±1.25*	*±10.5*
Wheat flour 650	***Avrg.***	***1,469***	***347***	***1.2***	***0.27***	***72.4***	***1.15***	***2.04***	***10.7***
	CI	*±20.12*	*±2.59*	*±0.14*	*±0.11*	*±0.82*	*±1.06*	*±1.01*	*±0.30*
Whole-grain wheat flour	***Avrg.***	***1,462***	***346***	***1.93***	***0.32***	***66***	***1.93***	***7.44***	***12.7***
	CI	*±41.88*	*±10.14*	*±0.28*	*±0.12*	*±3.04*	*±3.20*	*±0.82*	*±0.02*
Durum wheat flour	***Avrg.***	***1,487***	***350***	***1.65***	***0.37***	***69.4***	***2.05***	***5.57***	***13***
	CI	*±40.38*	*±10.58*	*±0.70*	*±0.11*	*±5.18*	*±1.76*	*	*±1.63*
Spelt wheat flour	***Avrg.***	***1,431***	***340***	***2.35***	***0.52***	***63.4***	***2.72***	***7.12***	***13.8***
	CI	*±50.23*	*±12.94*	*±0.50*	*±0.26*	*±5.44*	*±2.24*	*±2.21*	*±0.65*
Rye flour	***Avrg.***	***1,376***	***326***	***1.7***	***0.31***	***67.7***	***2.72***	***8.09***	***9.52***
	CI	*±50.58*	*±10.02*	*±0.29*	*±0.25*	*±2.59*	*±2.02*	*±3.50*	*±1.10*
Corn flour	***Avrg.***	***1,469***	***348***	***1.44***	***0.23***	***75.6***	***1.13***	***3***	***7.14***
	CI	*±11.28*	*±2.84*	*±0.31*	*±0.09*	*±1.09*	*±0.90*	*±1.14*	*±0.69*

3.2 Box plot analysis of the nutritional content of different flour categories

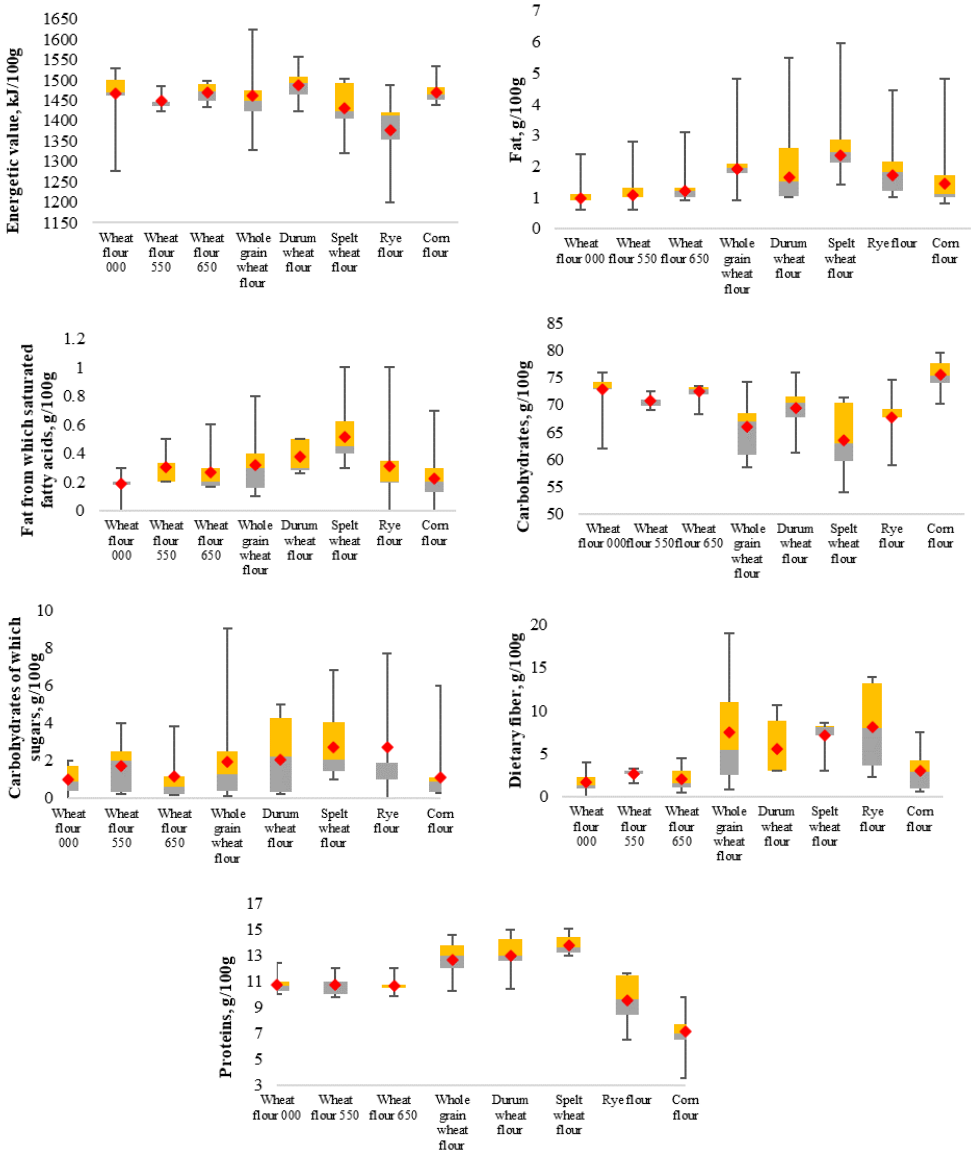
The box plot is an important tool to visualize and analyse the distribution of the values in the dataset. Thus, in order to provide a better insight into the characteristics of the flours analysed during this study, box plot analysis was used for the different types of flours, taking into consideration the most important parameters. As stated earlier, the median is statistically more representative than the mean values (*Williamson et al.*, 1989).

From the median energy values, it can be concluded that the studied flour types decrease in the following order: Durum wheat flour> Wheat flour 650> Wheat flour 000> Maize flour> Wholemeal wheat flour> Wheat flour 550> Spelt wheat flour> Rye flour (*Figure 1*). In terms of fat and saturated fatty acid content, it can be seen that the highest levels were found in whole-wheat flour, durum wheat flour, and spelt wheat flour. On the other hand, the lowest fat content was found in white wheat flour (000, 550, 650).

Furthermore, it is evident from the analysis that there are large differences between the highest and lowest values within the various product categories. The largest variation between the maximum and minimum energy content of the category was found for wholemeal flour (297 kJ/100 g), rye flour (288 kJ/100 g), and wheat flour 000 (251 kJ/100 g). The difference for spelt flour and durum flour was 183 kJ/100 g and 133 kJ/100 g respectively. The smallest differences were observed for maize (94 kJ/100 g), wheat flour 650 (63 kJ/100 g), and wheat flour 550 (60 kJ/100 g).

In all studied flour categories, the highest energy content was found in the case of *K classic* wholemeal wheat flour (1,625 kJ/100 g), while the lowest was identified for *Góbé* rye flour, with an energy content of 1,199 kJ/100 g. The individual values of the flours tested may be found in the supplementary materials, whereas the tables (S1–S8) summarize those values based on product categories, taking into account the following parameters: fat content, carbohydrate, protein, dietary fibre.

Regarding the fat content of flours, the lowest and the highest values were identified for *Auchan 000* wheat flour (0.6 g/100 g) and *Kenyérvarázs* corn flour (3.1 g/100 g) respectively. Since carbohydrates are present in the highest proportion in the flour, the samples were categorized based on this parameter, and the lowest carbohydrate content was found in the case of products under the *Economia* brand (54 g/100 g), while the highest carbohydrate content was found for the *Monidiferro* brand maize flour (79.55 g/100 g). On the other hand, the lowest protein concentration was observed for *Pronat* corn flour (3.6 g/100 g) and the highest for *Biopont* and *Dr. Avraham* (15 g/100 g). Furthermore, in terms of dietary fibre, the minimum value was observed for wheat flour 650 (0.4 g/100 g) and the maximum for rye flour (13.9 g/100 g) (see supplementary material tables S1–8).



Notes: The box plots were prepared using Microsoft Excel software. The lower and upper limits represent the second (50P) and third (75P) quartiles, the rhombus represents the average, and the ends of the whiskers represent the minimum and the maximum values.

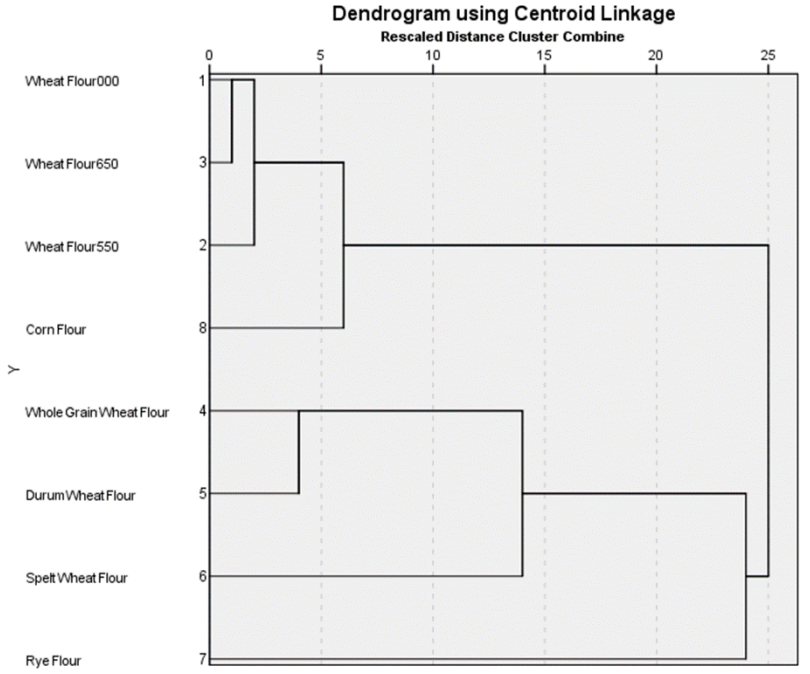
Figure 1. Box plot analysis of different flour categories

It has been shown that saturated fat intake increases heart disease risk factors, and hence the AHA (American Heart Association) recommends 5% to 6% of calories from saturated fat per day. The analysed saturated-fat-to-fat ratio in our case was on average 0.21, ranging from 0.16 (corn flour) to 0.28 (wheat flour 550). In terms of carbohydrate,

maize flour and white wheat flour have the highest carbohydrate content, with more than 70 g/100 g. Similar carbohydrate levels were observed for durum wheat (69.4 g/100 g), while spelt flour was characterized by low carbohydrate content (63.43 g/100 g) and high sugar content (2.72 g/100 g). The percentage of sugars in relation to total carbohydrates varied within the categories, the highest sugar content being found in flours with lower carbohydrate, particularly spelt (4.28%) and rye flour (4.02%). This type of negative correlation between carbohydrate, dietary fibre, and protein content has also been detected. Among the flours evaluated, wholemeal flour, durum, spelt, and rye flours have the highest protein and fibre content.

3.3 Hierarchical cluster analysis (HCA)

The aim of hierarchical cluster analysis is to create a dendrogram where the studied flours are grouped into branches that are close to each other based on their similarities. *Figure 2* depicts the hierarchical cluster analysis results as a dendrogram.



Note: The dendrogram was prepared using SPSS statistical software (centroid linkage, Euclidian distance).

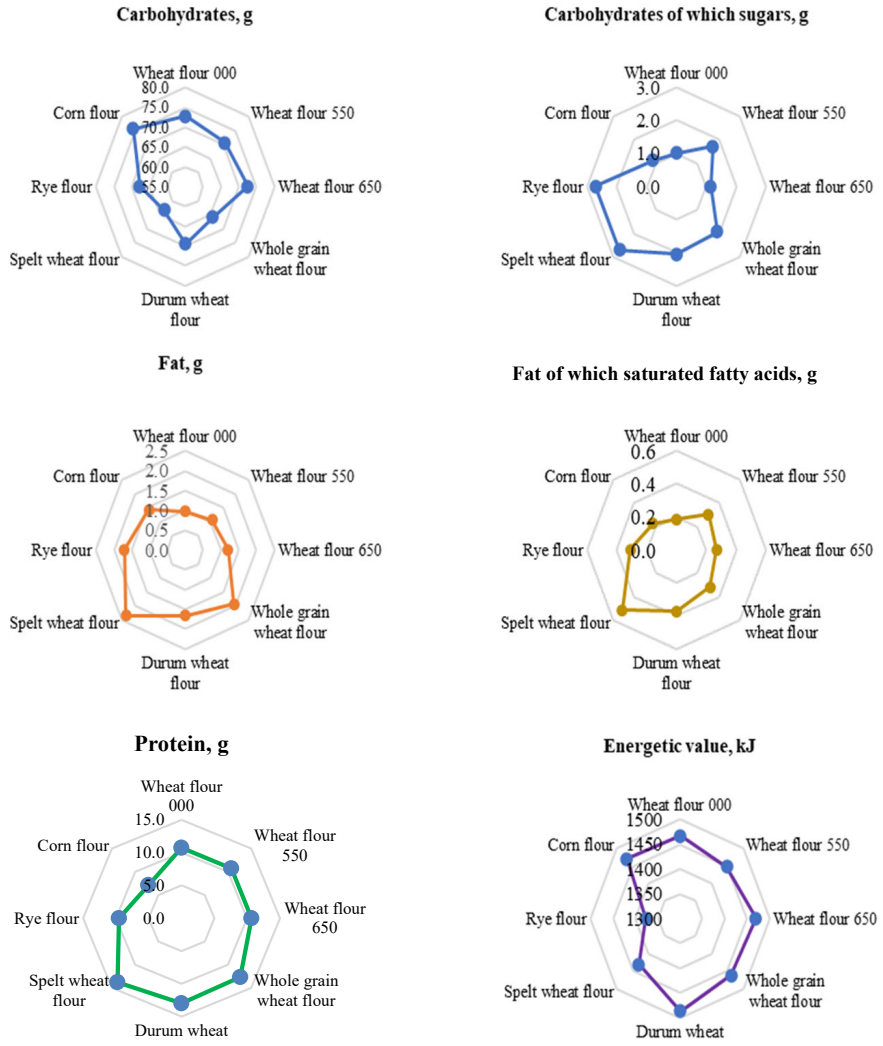
Figure 2. Cluster analysis of different types of flour

Based on the declared nutrition values, three distinct clusters can be identified. Wheat flour 000, 650, and 550 together with corn flour represent the first main cluster. In cluster two, whole-grain wheat flour and durum wheat flour are located most closely

in sub-cluster 2.1. Sub-cluster 2.2 contains only spelt wheat flour. Meanwhile, rye flour has different properties than the other flours and thus forms the third cluster.

3.4 Surface radar plot analysis

Similarly to the box plot analysis, an alternative visualization method was used to further illustrate the conspicuous properties. The purpose of surface radar plot graphs was to convey meaning to the various flour types through their difference by using surface radar plots.



Note: Plots were prepared using Microsoft Excel 2016.

Figure 3. Surface radar plots of the studied flours based on the composition

Data of the nutrition components (fat, saturated fatty acid, carbohydrate, sugar, dietary fibre, protein, energetic value) of the studied flours are presented separately (*Figure 3*). By analysing the composition of the groups, it is possible to clearly determine the order of the groups. Based on the carbohydrate content, the following increasing order was obtained (g/100 g): Spelt wheat flour (63) < Whole-grain wheat flour (66) < Rye flour (68) < Durum wheat flour (69) < Wheat flour 550 (71) < Wheat flour 650 (72) < Wheat flour 000 (72.8) < Corn flour (76). Similarly, the protein content shows the following order (g/100 g): Corn flour (7) < Rye flour (10) < Wheat flour 000 (10.7) < Wheat flour 550-650 (11) < Whole-grain and durum wheat flour (13) < Durum wheat flour (14).

3.5 Hypothesis tests

According to the hypothesis test results, differences were plotted out between the studied flour types. The summary of the t-test results are presented in *Table 3*. The detailed results are presented in the supplementary material tables (S9–15). The most significant differences were found in the case of spelt and rye flour (6 out of the 7 parameters) compared to the white flours.

Table 3. Summarized t-test results; the number of parameters that present the differences

	Wheat flour 550	Wheat flour 650	Whole-grain wheat flour	Durum wheat flour	Spelt wheat flour	Rye flour	Corn flour
Wheat flour 000	2	2	5	5	6	6	3
Wheat flour 550		1	4	2	4	3	2
Wheat flour 650			4	2	4	3	2
Whole-grain wheat flour				1	1	2	4
Durum wheat flour					1	2	3
Spelt wheat flour						2	5
Rye flour							4

4. Discussion

According to the results, it can be stated that there is a wide variety of flours on the Romanian market, and they show a very high variability regarding the nutrient content. The majority of the flour types examined (51.75%) were white

flours derived from wheat, while durum wheat flour (7%) and spelt wheat (7%) were present in smaller amounts. Only 13.27% of the products examined in this study were considered whole wheat, indicating that Romania's reform bakery is still in its early stages. Therefore, most of the bread and baked goods on the market are made from white wheat flour.

Whole-wheat flour has a high mineral content derived from the grain husk and a high fat content derived from the germ (*Wieser et al.*, 2020). Furthermore, low-ash white flours (types 000, 550, 650) are obtained from the endosperm (*Zafar et al.*, 2020).

According to similar research, wholemeal wheat flour, with its higher micronutrient content, plays an important role in human nutrition, with Mg^{2+} being an essential ion, containing at least 56.25 mg of magnesium per 100 g of food (*Ciudad-Mulero et al.*, 2021).

In order to have a healthy lifestyle, a key step is to eat a healthy diet, and therefore it is essential to promote these values. Regarding Romania, there is a major gap in the promotion of healthy eating, which favours a different lifestyle with lower carbohydrate and higher fibre flour (*Liu et al.*, 2020).

Furthermore, in terms of maize flour variability, it can be seen that it is present on the Romanian market with a diverse range of products, accounting for more than 20% of the studied products, which is not surprising given that corn flour is frequently used in the preparation of polenta in Romanian cuisine (*Laurentiu*, 2018).

The nutritional content of various grain flours is determined fundamentally by the varieties. Moreover, the variation in the quality of cereals depends on a wide range of factors influencing genotype and growing conditions (*Wieser & Kieffer*, 2001). There is an important trend in the baking industry worldwide to harmonize the benefits of different flour varieties in order to develop healthier products (*Dabija & Paius*, 2015; *Pontonio et al.*, 2021; *Valli et al.*, 2016).

5. Conclusions

In this study, we analysed the most commonly used cereal flours and their nutritional content available in the Romanian market, which is dominated by wheat flour because wheat is the most important cereal. Regarding the origin of the studied flour products, besides Romanian production, products from several EU Member States are also present on the Romanian market. Significant differences were found between the categories of the studied flours, with white wheat flour having a lower dietary fibre, fat, and protein content but a high energy and carbohydrate content. On the other hand, wholemeal, durum, spelt, and rye flours are lower in carbohydrate and higher in fibre and fat. Product labelling checks revealed inconsistencies, with many local producers failing to indicate

the fibre and salt content. Further studies are needed to determine the mineral composition of the flours in order to reveal the possible health effects and benefits.

Abbreviations

Avrg – average, 50P – median, 50P – 50 percentile, 75P – 75 percentile, CI – confidence interval, EU – European Union, GD – government decision, HCA – hierarchical cluster analysis, HCl – hydrochloric acid, n – sample size, N – number of samples, s – standard deviation, t – student t.

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Exploring the effects of comminution level and natural antioxidant incorporation on the quality and oxidative stability of turkey meat system

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Abstract. This study aimed to explore the effects of different comminution degrees and the incorporation of a natural antioxidant on the quality attributes and oxidative reactions of turkey meat. Four distinctive turkey meat systems were established, namely: 3 mm minced treatment (M), 3 mm minced treatment with the addition of 200 ppm gallic acid equivalent *Aloe vera* (*Aloe barbadensis* Mill.) extract (MA), fine-ground treatment (FM), and fine-ground treatment with the addition of 200 ppm gallic acid equivalent *Aloe vera* extract (FMA). The evaluation encompassed an in-depth analysis of various quality parameters and the assessment of lipid-protein oxidation reactions throughout the storage period. The inclusion of *Aloe vera* extract (AE) increased the pH and b* values while simultaneously decreasing the L* and a* values. Conversely, increasing the degree of comminuting manifested an elevation in L* values, concomitant with a decline in a* values. Increased comminuting degree ratios were found to contribute to an exacerbation of oxidative reactions. Nonetheless, the strategic utilization of AE demonstrated its potential to effectively mitigate oxidative reactions during storage.

Keywords and phrases: turkey meat, comminution, *Aloe vera* extract, oxidative reactions, quality parameters, breast muscle

1. Introduction

Meat has been a fundamental source of nutrition for humans throughout history (*Geiker et al.*, 2021). Nowadays, an increasing number of people recognize that turkey meat, classified as poultry, is more economical and contains less fat than red meat (breast fillet and drumstick approximately 1% and 8% resp.). This has

led to a rise in the consumption of turkey meat as a dietary staple. Due to its high levels of polyunsaturated fatty acids, B vitamins, and low cholesterol, many nutritionists advocate the incorporation of turkey meat into our diet. Consequently, the demand for poultry and chicken products has substantially increased over the last decade (*Bashiry et al.*, 2021).

Global poultry meat consumption has significantly increased in recent years, with poultry meat accounting for over 35% of total meat consumption, according to the FAO. In 2020, global poultry meat consumption reached 134.5 million tons, representing 35.6% of the world's meat consumption (*FAOSTAT*, 2023). Turkey meat, classified as poultry, has gained popularity due to its lower fat content and affordability. Turkey has seen a rise in turkey meat production in line with this trend. However, the per capita turkey meat consumption in Turkey lags behind that of major consumers such as the United States and the European Union (*Koyubenbe & Konca*, 2010). This suggests potential for further growth in the meat industry influenced by cultural and economic factors affecting dietary habits.

Foods derived from animal muscles, including fish, red meat, and poultry, are prone to lipid oxidation. Factors such as size reduction techniques, exposure to oxygen, and the generation of reactive oxygen species during processes such as heating, salting, and high-pressure treatment contribute to this oxidation process (*Estévez*, 2021). Mincing or comminution increases the surface area of the meat, exposing it to more oxygen and potentially accelerating oxidation. Enzyme release triggered by mincing also plays a role in oxidation (*Veberg et al.*, 2006). Various external and internal factors, such as fatty acid profile, fat content, temperature, light, moisture, atmospheric oxygen, and the presence of iron, activators, and inhibitors, also impact lipid oxidation (*Abeyrathne et al.*, 2021). The primary impacts of oxidation processes on meat are often related to its sensory characteristics, including alterations in colour, texture, and appearance, as well as the development of off-flavours and off-odours. These changes can result from colour loss, texture damage, and the formation of undesirable rancid flavours caused by the degradation of lipids and proteins in the meat (*Echegaray et al.*, 2021).

Antioxidants are used to delay or prevent the negative consequences of oxidative changes in food. Concerns over the toxicity of synthetic chemicals have led to a search for natural antioxidant sources. The demand for healthier products has driven the food industry to seek natural alternatives to chemical additives. Plant extracts and essential oils from various sources, such as aromatic plants, leaves, seeds, and spices, can serve as natural antioxidants in meat products. They effectively delay or inhibit lipid and protein oxidation without compromising sensory or nutritional properties. Moreover, they minimize rancidity and extend the shelf life of meat products by preventing oxidative chain reactions (*Kumar et al.*, 2015; *Pateiro et al.*, 2021).

Aloe vera is a versatile plant that has been used for its medicinal properties for thousands of years. It is widely used in the cosmetic as well as food industries due to its numerous benefits. *Aloe vera* is a natural antioxidant that has recently gained popularity in the food industry because it contains bioactive compounds such as polysaccharides, glycoproteins, or vitamins, such as A, C, E, carotenoids and phenolic compounds, delaying lipid oxidation in the food industry as well as providing health benefits to the consumer (*Hęś et al.*, 2019). *Aloe vera* is utilized in various forms of dietary products, including gel, essential oil, leaves, and extracts. As a result of the abundance of bioactive compounds present in *Aloe vera*, numerous food processors are currently focusing on developing functional meat products that incorporate *Aloe vera* (*Biswas et al.*, 2014). *Aloe vera* has been utilized in various meat products such as burger *(Soltanizadeh & Ghiasi-Esfahani*, 2015), nugget (*Bhat et al.*, 2015; *Rajkumar et al.*, 2016), meat rolls (*Rathour et al.*, 2019), and dry fermented sausage (*Uşan et al.*, 2021). While there is a singular study on the utilization of *Aloe vera* extract in turkey meat (*Biswas et al.*, 2014), it does not examine the potential influence of using *Aloe* extract in minced turkey meat prepared at varying degrees of mincing. In the light of this information, the purpose of the study was to take a closer look at the quality parameters and oxidative changes that occur when *Aloe vera* extract is used in the formulation of various mincing degrees of turkey breast meat.

2. Materials and methods

2.1 Materials

Turkey breast meat with a moisture content of 75%, protein content of 21.6%, lipid content of 2.4%, and ash content of 1.1% was procured from Migros Trading Co., Ltd. (Izmir, Turkey) while maintaining the cold chain during transportation to the laboratory. *Aloe vera* extract (2.67 mg GAE/g) was obtained from Nurbal Healing Centre (Istanbul, Turkey). Sous-vide cooking bags were supplied by Fitpak Packaging and Chemistry Trade Inc. in Manisa. All chemical reagents used in the study were procured from Sigma–Aldrich Chemical Co. (St. Louis, USA).

2.2 Experimental design

The present study was designed to investigate the impact of different degrees of comminution and the addition of *Aloe vera* extract on turkey breast meat. Four batches were produced and grouped as follows: the first group (M) consisted of turkey breast meat minced through a 3 mm plate without the addition of *Aloe vera*

extract (control treatment-1). The second group (FM) involved finely grounded turkey breast meat in a Thermomix (Vorwerk, Germany) without the inclusion of *Aloe vera* extract (control treatment-2). The third group (MA) included turkey breast meat minced through a 3 mm plate with the addition of *Aloe vera* extract (200 ppm gallic acid equivalent, 0.75 g/kg breast meat). Lastly, the fourth group (FMA) consisted of finely grounded turkey breast meat with *Aloe vera* extract coded as FMA (200 ppm gallic acid equivalent, 0.75 g/kg breast meat).

2.3 Preparation of turkey meat systems

In this study, turkey breast meat was minced using a household meat grinder (Arçelik, Turkey) equipped with a 3 mm diameter plate. The resulting minced meat was then divided into two equal parts. Half of the samples were treated with 0.75 g of *Aloe vera* extract per kilogram of meat, while the other half served as control-1.

To produce FM (control-2) and FMA (*Aloe vera* extract added), the minced meat was homogenized in a Thermomix (2500 rpm, 45 s) and then divided into two parts. Half of the homogenized samples were treated with 0.75 g of *Aloe vera* extract per kilogram of meat. All experimental batches were divided into smaller portions and shaped with metal plates for sampling at different storage periods and packaging conditions.

The samples were vacuum-packed (PA + PE, 160 cm^3/m^2/day oxygen permeability) and subjected to sous-vide cooking at 70°C for 30 minutes, followed by cooling to ambient temperature. The cooked samples were then stored at 4°C for 3, 6, and 9 days prior to analysis.

2.4 pH

The pH values were measured by immersing them in 3 different points with the dipping tip of the pH meter (WTW pH 330i/SET).

2.5 Colour measurement (L*, a*, b*)

The brightness (L*), redness (a*), and yellowness (b*) colour parameters of the samples were assessed using a portable colour measurement device (CR-400, Konica Minolta, Japan). The surface colour of the samples was measured using a portable colorimeter (CR-200, Konica Minolta, Japan) equipped with a ten-degree observer angle and D65 illuminant. The Chroma (C*) and Hue angle (h*) were determined, and the Euclidean distance (ΔE) between samples was calculated following the guidelines set by the American Meat Science Association (AMSA 2012).

Chroma angle: $C^* = \sqrt{a^{*2} + b^{*2}}$ (1)

Hue angle: $h^* = \arctan\left(b^*/a^*\right)$ (2)

Euclidean distance: $\Delta E = \sqrt{(L^*_S - L^*_T)^2 + (a^*_S - a^*_T)^2 + (b^*_S - b^*_T)^2}$ (3)

S representing standard and T representing treated were calculated according to the American Meat Science Association guidelines (AMSA 2012).

2.6 Water-holding capacity

The moisture retention capacity of the uncooked product was evaluated by *Hughes et al.* (1997) using the method specified by modifications.

2.7 Expressible moisture

The fold wrap method described by *Earl et al.* (1996) was used to determine the expressible moisture (EM) of the samples. This involved placing 1.5 g of the sample onto a thimble of filter papers, which were then subjected to ultracentrifugation at 14,000 rpm (18,407 g) for 15 minutes at 4°C. The amount of moisture absorbed by the filter papers was then used to calculate the EM.

$$\text{EM (\%)} = \frac{Weight\ of\ the\ moisture\ expressed}{Original\ weight\ of\ the\ sample} \cdot 100 \quad (4)$$

2.8 Lipid oxidation

Peroxide number and TBARS (thiobarbituric acid reactive substance) analyses were conducted in accordance with *AOAC* (2012) and *Witte et al's* (1970) methods respectively.

2.9 Total sulfhydryl content

The total amount of sulfhydryl (thiol) of samples was determined according to *Eymard et al.* (2009) and *Ellman*'s (1959) method. Samples were mixed with 0.05 M potassium phosphate buffer and homogenized for 1 min by using ultra-turrax (Ultra-Turrax, IKA, Germany). The resulting mixture was centrifuged (Sigma, 3-30 KS, Germany) at 14,000 rpm (18407 g), and the upper phase was taken. To this, a solution of DTNB was added, and the mixture was incubated at 40°C for 15 min. The absorbance values of the sample and blind were read at 412 nm using a spectrophotometer, and the sulfhydryl amount was calculated using a

molar absorption coefficient of 13.600 $M^{-1}.cm^{-1}$. The results were reported as nmol sulfhydryl/mg protein.

2.10 Statistical analyses

The experiment was repeated on two different days, resulting in two distinct batches. Measurements for the relevant characteristics were conducted three times for each batch, and the average values for the measured parameters were calculated. These average values were compared using analysis of variance (ANOVA) performed with SPSS software for Windows (version 21.0, SPSS Inc., Chicago, IL, USA). The production batches were considered as a random factor, while each treatment was regarded as a fixed factor. To analyse the storage data, a two-way ANOVA was employed, considering treatment and storage time as the main factors. Duncan's multiple range test was applied to compare the means of different groups, with a significance level set at $P < 0.05$.

3. Results and discussion

3.1 Water-holding capacity and expressible moisture

Water-holding capacity (WHC) values of samples ranged from 57.55% to 63.14% (*Table 1*). The interaction between *Aloe vera* extract and the degree of comminution significantly affected the WHC in turkey meat. While reducing the particle size of turkey meat with *Aloe vera* extract had a potentially negative impact on WHC, incorporating *Aloe vera* extract improved the WHC of coarsely ground meat ($P < 0.05$). This can be explained by the fact that as comminution increases, the ability of turkey breast meat to retain water decreases. Myofibrillar proteins, particularly myosin, play a key role in WHC. Denaturation of myosin is suggested to contribute to a decline in WHC (*Bowker & Zhuang*, 2015). Interestingly, the MA group showed the highest WHC value (*Table 1*). *Aloe vera*-derived polysaccharides, alone or in combination with proteins, have been found to enhance water-holding capacity by creating a network structure that effectively retains water. Similar findings have been reported in burger formulations with *Aloe vera* powder (*Soltanizadeh & Ghiasi-Esfahani*, 2015).

Lower water-holding capacity in meat is typically correlated with higher expressible moisture content. The present study provides insights into the expressible moisture values of the meat systems, as presented in *Table 1*. The EM values for the turkey meat systems ranged from 11.65% to 14.59%. Notably, the utilization of *Aloe vera* extract and the degree of comminution exhibited significant effects on

the EM values ($P < 0.05$). Interestingly, the incorporation of *Aloe vera* extract did not induce any substantial changes in the water-holding capacity of coarse ground meat systems. However, in finely ground meat, the addition of the extract led to a decrease in EM. Among the treatments, the M demonstrated the highest EM value ($P < 0.05$), while the MA, FM, and FMA treatments exhibited similar values. The inclusion of *Aloe vera* powder in beef burgers has previously been shown to increase moisture retention, potentially attributed to the extract's ability to enhance the water-holding capacity of the product (*Soltanizadeh & Ghiasi-Esfahani*, 2015). This finding aligns with a separate study on meat analogues, where samples incorporating Spirulina flour exhibited higher expressible moisture content (*Palanisamy et al.*, 2019). In addition, *Bhat et al.* (2015) also showed that nuggets formulated with *Aloe vera* pulp had enhanced emulsion stability and cooking yield.

Table 1. Water-holding capacity and expressible moisture

Treatment	Water-holding capacity (%)	Expressible moisture (%)
M	$59.55^{bc} \pm 1.79$	$14.59^{a} \pm 1.00$
MA	$63.14^{a} \pm 0.82$	$14.51^{a} \pm 1.54$
FM	$59.98^{b} \pm 0.97$	$11.65^{b} \pm 0.13$
FMA	$57.55^{c} \pm 1.09$	$13.59^{a} \pm 0.81$

Notes: $^{a–c}$ – different letters in the same row indicate a significant difference ($P < 0.05$). Data were presented as the mean ± standard deviation. M: 3 mm minced; MA: 3 mm minced + AE; FM: finely grounded; FMA: finely grounded + AE.

3.2 pH

The pH value of the meat systems was significantly influenced by the incorporation of *Aloe vera* extract and the degree of comminution (*Figure 1*). Initially, the pH values ranged from 6.08 to 6.20, and by the end of the storage period, they reached a range between 5.86 and 6.00. Throughout the entire storage period, pH values exhibited an increasing trend with the addition of *Aloe vera* extract ($P < 0.05$). However, *Uşan et al.* (2021), *Bhat et al.* (2015), and *Shahrezaee et al.* (2018) reported a decrease in meat products added with *Aloe vera* extract, *Aloe vera* pulp, and *Aloe vera* gel powder. These controversial results regarding pH can potentially be attributed to the sous-vide cooking technique employed and the presence of the added extract. It is believed that the complex relationship between thermal processing and the structural transformations of the *Aloe vera* extract may explain the divergent pH responses observed in this study compared to prior research. As the degree of comminution increased, the pH value of the meat systems decreased ($P < 0.05$). Remarkably, the FM treatment exhibited the

lowest pH value throughout the entire storage period ($P < 0.05$). After the 6th day of storage, the pH values of all treatments displayed a decline, which could be attributed to the microbial spoilage.

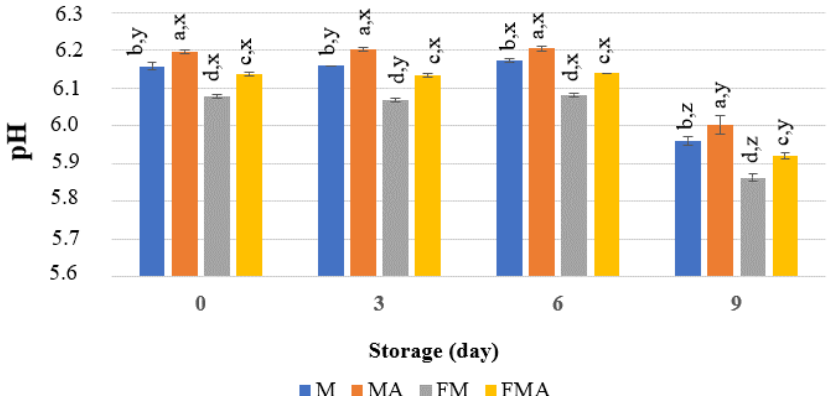

Figure 1. pH values (sample codes are given in *Table 1*)

3.3 Colour

Initially, the L* values ranged between 71.62 and 75.48 (*Figure 2*), with the lowest values measured in the MA group at the beginning of the storage period. The L* values of the other treatments showed similar results. The application of AE resulted in a decrease in the lightness of the meat system on all storage days except the first day. During the storage period, no significant changes in L* values were observed except for a decrease observed in the FMA group on the 9th day. By the end of the storage period, the L* values ranged between 71.52 and 75.36. Furthermore, an increase in the degree of comminution led to an increase in L* values at the end of storage, with the FM group displaying the highest value. This phenomenon can be attributed to the assumption that an increase in the degree of comminution results in a decrease in water-holding capacity. As a result, water tends to rise to the surface, causing enhanced light reflection and increased lightness due to the presence of trapped air bubbles.

On Day 0, the a* values ranged from 1.93 to 3.76 (*Figure 2*). The highest a* value was observed in group M. Both the addition of AE and an increase in the degree of comminution had a reducing effect on the a* values. Throughout the storage period, samples with a higher degree of comminution displayed lower a* values. Among the treatments, the FMA group, which included the addition of AE and fine mincing, consistently exhibited the lowest a* values. This can be attributed to the green colour of AE, which is derived from the whole leaf and has been found

to reduce both the a* and L* values. During the storage period, the a* values of the M and FMA treatments remained unchanged, while those of the MA and FM treatments increased. By the end of the storage period, the a* values ranged between 2.22 and 4.21. A similar trend observed at the beginning of storage was also noted among the groups on the 9th day.

The b* values were influenced by the degree of comminution and the addition of *Aloe vera* extract. Initially, the b* value ranged between 10.59 and 12.85. Throughout the storage period, the MA and FMA groups, which included the *Aloe vera* extract, exhibited the highest b* values. The effect of the comminution degree on b* value was not significant at the beginning of storage. During the storage period, the b* values increased in the M and FMA, while fluctuations were observed in the other treatments. By the end of the storage period, an increase in the degree of comminution led to a decrease in the b* value. The highest yellowness was measured in the FMA where AE was incorporated to the formulation. In a study involving goat meat, it was observed that the addition of *Aloe vera* resulted in an increase in b* values (*Rathour et al.*, 2019). *Uşan et al.* (2021) also stated that the use of *Aloe vera* extract in dry fermented sausage resulted in higher b* values, lower L* and a* values.

Chroma, which represents the saturation or intensity of meat colour, is directly influenced by the concentration and status of myoglobin. The Chroma values observed in this study ranged between 11.05 and 13.20 (*Table 2*). The comminution degree had a significant impact on Chroma values, with higher degrees of comminution resulting in lower Chroma values. Conversely, the use of *Aloe vera* extract had the opposite effect on the meat systems, leading to higher Chroma values ($P < 0.05$). The FM treatment exhibited the lowest Chroma value, indicating greater discolouration in the colour of this treatment ($P < 0.05$).

The Hue angle index (H°) is a measure of colour development from red to yellow, where larger angles indicate lower redness. Our findings indicate that the inclusion of *Aloe vera* extract led to increased Hue angle values (*Table 2*). Specifically, minced turkey meat systems treated with *Aloe vera* extract (MA and FMA) exhibited higher Hue angles compared to other treatments ($P < 0.05$), suggesting that these treatments had less redness due to the natural colour of the extract. The degree of comminution did not significantly affect the redness of the meat systems ($P > 0.05$). Moreover, the M and FM treatments showed similar Hue angle index values, as did the MA and FMA treatments. In a study, where *Aloe vera* extract was added to chevon rolls, lower Hue angles and higher Chroma values were reported (*Rathour et al.*, 2019).

The total colour difference value (ΔE) serves as a useful indicator of the impact of various applications on the colour of meat products. For the human eye to perceive a noticeable colour difference, the threshold value is generally considered to be between 2 and 6 (*Larraín et al.*, 2008). In our study, the total colour differences ranged from 1.34 (FM) to 3.66 (MA). Based on these findings, it can be concluded

that the effect of excessive reduction in the particle size of turkey meat on the colour was almost imperceptible when compared to the addition of *Aloe vera* extract. The differences became detectable when AE was incorporated into the formulation.

Table 2. Turkey meat system colour indices on Day 0

Treatment	Colour indexes		
	Chroma	Hue angle	ΔE
M	$11.80^{d} \pm 0.26$	$71.43^{bc} \pm 1.58$	-
MA	$13.20^{b} \pm 1.57$	$76.80^{a} \pm 0.69$	$3.66^{a} \pm 1.10$
FM	$11.05^{c} \pm 0.13$	$73.44^{c} \pm 0.51$	$1.34^{c} \pm 0.44$
FMA	$12.78^{a} \pm 0.67$	$81.26^{ab} \pm 1.10$	$2.49^{b} \pm 0.42$

Notes: $^{a–d}$ – different letters in the same row indicate a significant difference ($P < 0.05$). Data were presented as the mean ± standard deviation. Sample codes are given in *Table 1*.

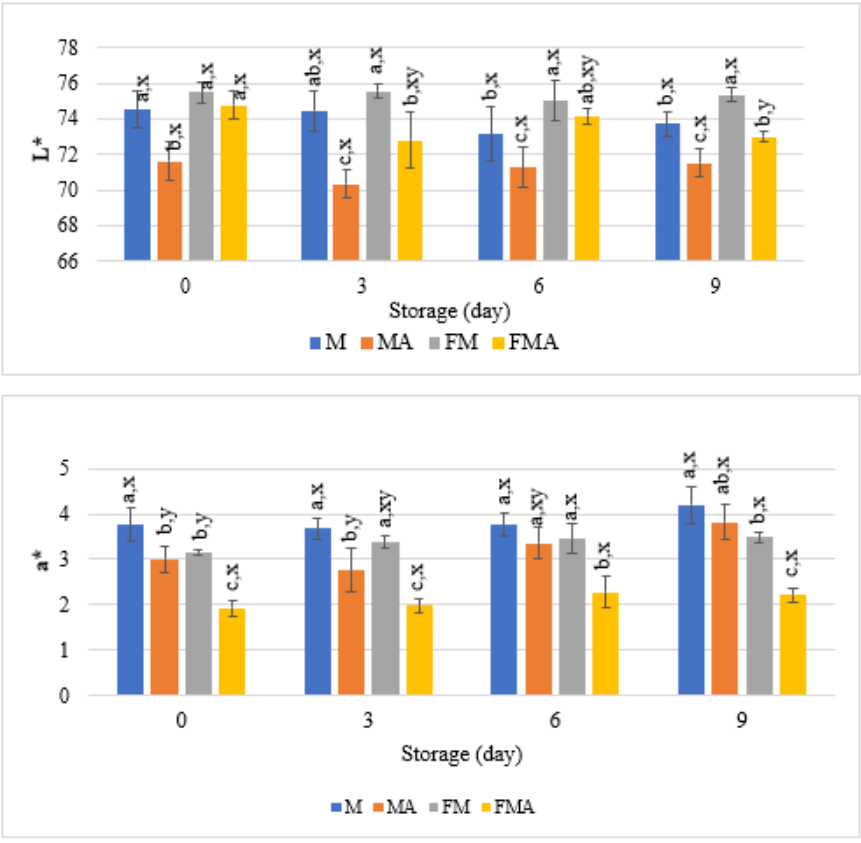

Figure 2a. Colour parameters (sample codes are given in *Table 1*)

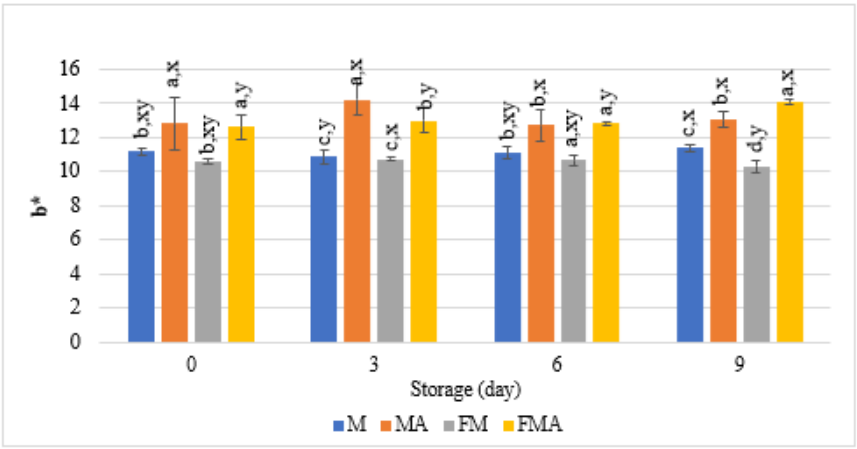

Figure 2b. Colour parameters (sample codes are given in *Table 1*)

3.4 Peroxide value

During the initial stages of lipid oxidation, radical peroxide and hydroperoxides are the compounds primarily responsible for the process of primary oxidation. As the storage period progresses, these compounds undergo transformation into secondary oxidation products (*Serdaroğlu et al.*, 2022). The initial peroxide values of the treatments in our study ranged from 0.78 to 1.96 meqO_2/kg (*Table* 3). The lowest peroxide value was observed in the MA treatment, and no significant differences were observed among the peroxide values of the other treatments. The degree of comminution did not significantly affect the peroxide values during the first 6 days of storage. However, on the 6th day of storage, the highest peroxide values were observed in all samples ($P < 0.05$). The use of *Aloe vera* extract was found to be particularly effective in samples that underwent 3 mm mincing during the later stages of storage. Similarly, turkey meat treated with bioactive compounds from *Aloe vera* gel had lower peroxide values than control groups (*Biswas et al.*, 2014). Also, the inclusion of pomegranate peel extract resulted in a decrease in peroxide values in ground buffalo meat (*Ghimire et al.*, 2022). Fluctuations in peroxide values were observed throughout the storage period, and by the 9th day, the values reached 1.37–2.40 meqO_2/kg. This can be attributed to the accumulation of secondary oxidation products in the environment, as hydroperoxides are converted to aldehydes and ketones (*Ghimire et al.*, 2022). The M treatment exhibited the highest peroxide value at the end of the storage period ($P < 0.05$), while no significant differences were observed among the other treatments.

3.5 TBARS

The initial TBARS values of cooked turkey meat systems ranged from 0.61 to 0.93 mg malonaldehyde/kg (*Table 3*). The comminution degree did not have a significant effect on the initial TBARS values; however, the inclusion of *Aloe vera* extract resulted in a reduction of TBARS in the 3 mm minced treatment. The lowest TBARS value was observed in the MA treatment, while no statistical differences were observed among the other treatments ($P > 0.05$).

During the storage period, the TBARS values exhibited fluctuations. The use of *Aloe vera* extract during the later stages of storage significantly slowed down oxidation ($P < 0.05$) or did not create any significant differences compared to the control treatments. The highest lipid oxidation was observed on the 9th day of storage in all treatments. The accelerating effect of excessive reduction in particle size was more pronounced at the end of the storage period (FM); however, the inclusion of *Aloe vera* extract was able to reduce the TBARS values in this treatment ($P < 0.05$). The presence of antioxidant compounds, such as vitamins C and E, beta-carotene, flavonoids, and phenolic compounds in *Aloe vera*, may be the main reason for the delay in oxidation (*Hęś et al.*, 2019). Similar results were reported by *Bhat et al.* (2015) in chicken nuggets added with 5%, 10%, and 15% *Aloe vera* pulp. *Uşan et al.* (2021) also reported that *Aloe vera* extract could effectively retard oxidation in sausages. Also, different forms of *Aloe vera* have successfully retarded lipid oxidation in goat meat nuggets (*Rajkumar et al.*, 2016), turkey meat (*Biswas et al.*, 2014), and raw meat batter (*Kumar & Langoo*, 2016).

Table 3. Lipid oxidation of turkey meat systems

Peroxide values ($meqO_2$/kg)	**Storage (day)**			
	0	**3**	**6**	**9**
M	$1.77^{a,yz} \pm 0.26$	$1.26^{a,z} \pm 0.30$	$3.05^{ab,x} \pm 0.13$	$2.40^{a,xy} \pm 0.32$
MA	$0.78^{b,z} \pm 0.01$	$1.39^{a,y} \pm 0.20$	$2.17^{c,x} \pm 0.38$	$1.58^{b,y} \pm 0.79$
FM	$1.27^{ab,y} \pm 0.11$	$0.89^{a,z} \pm 0.10$	$3.31^{a,x} \pm 0.30$	$1.37^{b,y} \pm 0.20$
FMA	$1.96^{a,xy} \pm 0.83$	$1.39^{a,y} \pm 0.40$	$2.68^{bc,x} \pm 0.09$	$1.39^{b,y} \pm 0.28$
TBARS (mg malonaldehyde/kg)				
M	$0.86^{a,z} \pm 0.04$	$0.76^{b,z} \pm 0.13$	$1.25^{a,y} \pm 0.07$	$2.85^{b,x} \pm 0.29$
MA	$0.61^{b,y} \pm 0.05$	$0.69^{b,y} \pm 0.09$	$0.36^{d,z} \pm 0.01$	$3.00^{b,x} \pm 0.27$
FM	$0.93^{a,z} \pm 0.01$	$1.49^{a,y} \pm 0.05$	$0.74^{b,z} \pm 0.02$	$4.13^{a,x} \pm 0.38$
FMA	$0.91^{a,y} \pm 0.04$	$0.68^{b,z} \pm 0.11$	$0.56^{c,z} \pm 0.10$	$3.01^{b,x} \pm 0.10$

Notes: $^{a-c}$ / $^{x-z}$ – different letters in the same row/column indicate a significant difference ($P < 0.05$). Data were presented as the mean ± standard deviation (sample codes are given in *Table 1*).

3.6 Sulfhydryl content

Figure 3 represents the loss of sulfhydryl groups in the meat systems during storage. On Day 0, the sulfhydryl concentration of the various treatments varied from 2.93% (FMA) to 15.39% (M). The use of *Aloe vera* extract and the particle size of turkey meat had a significant effect on the total sulfhydryl groups ($P < 0.05$). The lowest total sulfhydryl content was observed in the FMA treatment, which consisted of finely ground turkey meat with *Aloe vera* extract.

Throughout the entire storage period, the samples treated with *Aloe vera* extract showed lower sulfhydryl groups except on the 3rd and 6th days of storage in the coarse-ground samples. The decrease in total sulfhydryl groups can be attributed to the formation of disulphide bonds and tyrosine as a result of oxidation and the cross-linking of sulfhydryl groups both within and between molecules.

By the end of the storage period, the sulfhydryl content ranged from 3.42% (MA) to 19.61% (FM). The highest value was observed in the FM, which consisted of fine-ground turkey breast meat with *Aloe vera* extract. Similarly, pomegranate peel extract, rosemary and lemon balm extracts, clove extract, wild thyme by-products extract, and purslane extract have also been reported to protect sulfhydryl groups in muscle foods due to their bioactive compounds (*Šojić et al.*, 2020; *Wang et al.*, 2021).

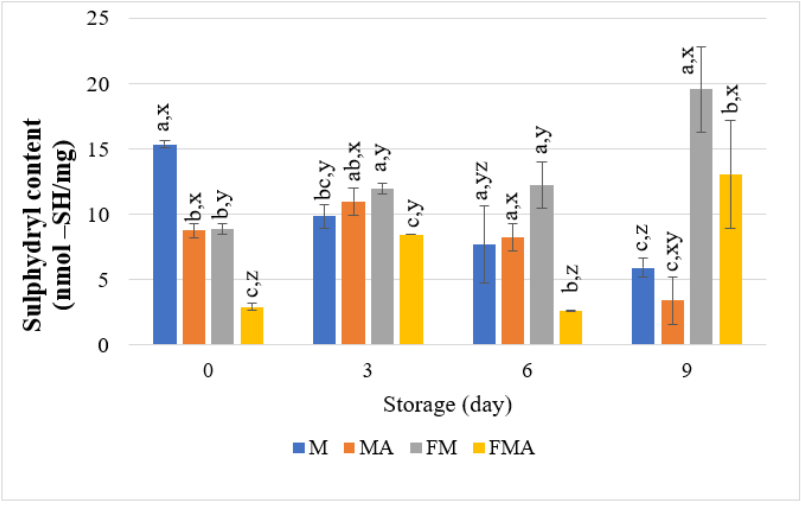

Figure 3. Sulfhydryl content (sample codes are given in Table 1)

4. Conclusions

It can be concluded that there exists a potential risk of progressed oxidation with an escalation in the reduction in the particle size of turkey meat. Nevertheless, the utilization of *Aloe vera* extract in the short-term storage of minced turkey meat demonstrates a promising capability to retard the process of oxidation during storage while concurrently averting the emergence of any discernible quality detriments. Further research in the field should focus on elucidating the specific mechanisms of antioxidant action of *Aloe vera* extract in minced meat products, determining the optimal concentration and application methods for achieving prolonged antioxidant activity and assessing its long-term storage stability. Comparative studies with other natural antioxidants and investigations into consumer perception and acceptance are also necessary to evaluate the potential market viability of *Aloe-vera*-treated minced meat products. By addressing these research areas, we can deepen our understanding of the protective effects of *Aloe vera* extract and develop innovative strategies to enhance the quality and shelf life of minced turkey meat while minimizing oxidative deterioration.

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Proximate composition and sensory acceptability of cowpea-based pudding produced from cowpea cultivated using different weed control methods

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Abstract. This study evaluates the effect of different weed control methods on the proximate composition and sensory properties of cowpea-based pudding produced from cowpea flour. Cowpea seeds of Ife Brown variety with three different treatments [(supplementary hoe weeding at 6 weeks after sowing, two hoe weeding at 3 and 6 weeks after sowing, and three hoe weeding at 3, 6, and 9 weeks after sowing (WAS)] were processed into flour samples and analysed for physicochemical properties using standard methods. The puddings were prepared from cowpea flour and were subjected to proximate composition, colour properties as well as sensory qualities using standard methods. The pH, total titratable acidity, water absorption capacity and amylose of cowpea flour were 4.85–5.10, 0.02%–0.05%, 276.00%–287.09%, and 22.04%–24.60% respectively. The ranges of values for moisture content, crude fat, total ash, crude fibre, crude protein, and total carbohydrate of cowpea based pudding were 74.26%–76.15%, 0.63%–0.71%, 0.75%–0.94%, 0.66%–0.75%, 16.70%–17.83%, and 5.11%–5.55% respectively. The colour properties of cowpea-based pudding were significantly affected ($p < 0.05$) by each treatment. However, cowpea-based pudding prepared from treatment of supplementary hoe weeding at 6 weeks after sowing and three hoe weeding at 3, 6, and 9 weeks after were preferred most by the panelist.

Keywords and phrases: cowpea, cowpea-based pudding, weed control

1. Introduction

Cowpea, also called black-eyed pea or southern pea, and botanically named Vigna unguiculate, is an annual crop belonging to the pea family and grown for its edible legumes (*Encyclopedia Britannica*, 2022). Cowpea is native to West Africa and cultivated around the world. They are crops that have compound leaves with three leaflets with climbing or trailing vines. The flowers of the plant usually grow in pairs or threes at the tip of long stalks and are usually of white, purple, or pale yellow colour. Depending on the cultivar type, their pods are usually long with cylindrical shape growing up to 20-30 cm. Cowpeas can grow in a wide range of soils and can develop well under water stress (*Dugje et al.*, 2009). It is a staple food that contributes to human nutrition with large amounts of proteins, carbohydrates, dietary fibres, vitamin of the B-complex, essential minerals, and a small quantity of lipids, and it has lower levels of anti-nutritional factors (*Nassourou et al.*, 2016; *Mtolo et al.*, 2017). Cowpea possesses anti-diabetic, anti-cancer, antihyperlipidemic, anti-inflammatory, and anti-hypertensive properties (*Kapravelou et al.*, 2015). Cowpea seeds are consumed boiled either alone or in combination with other foods such as rice, maize, and plantain. According to *Henshaw et al.* (2000) and *Idowu et al.* (2017), cowpeas could be processed into paste or flour for the preparation of various traditional foods such as *akara* (fried cowpea paste), *moin-moin* (steamed cowpea paste), and *gbegiri* (cowpea soup).

Despite cowpea having a wide potential as both a domestic and commercial crop and can be grown in a wide range of soil types, a number of constraints limit its production (*Edokpolor & Beckley*, 2019). These constraints include a lot of factors, especially weeds (*Adusei et al.*, 2016), which are unwanted plants that compete for light, space, soil nutrients, and carbon dioxide, thereby reducing crop yield. Weeds reduce crop yield by releasing allelopathic compounds into the environment (*Fragasso et al.*, 2013; *Marinov-Serafimov et al.*, 2019), thereby providing a conducive environment for pest and virus (*Fisichelli et al.*, 2014). Weeds impose a major constraint on crop production globally, losses caused by weeds alone in cowpea production ranging from 25% to 76% depending on the cultivar and the environment (*Adigun et al.*, 2014; *Gupta et al.*, 2016; *Osipitan et al.*, 2016; *Ugbe et al.*, 2016). Shortcomings brought about by the presence of weeds in cowpea production include reduction in crop yield, less efficient land use, higher cost of production due to insects and plant disease control, reduction in crop quality, water management problems, and less efficient utilization of labour (*Patil et al.*, 2014; *Gatachew et al.*, 2015; *Prabhu et al.*, 2015; *Singh & Sairam*, 2016).

Cowpea-based pudding, indigenously called *Ekuru* or white moin-moin, is a native meal in the south-western part of Nigeria, and it serves as a nutritional food in various cultural, traditional, and religious settings, especially among the

Yoruba people in Nigeria (*Olaleye et al.*, 2018). It is similar to steamed cowpea paste (moin-moin), as both are made from peeled cowpeas only; unlike cowpea-based pudding, steamed cowpea paste (moin-moin) is mixed with pepper and other ingredients before steaming. Cowpea-based pudding is served with fried pepper stew and then mashed up with pepper stew, while some people also enjoy the pudding with fermented maize pudding (*Agidi* or *Eko*), which is also cereal-based food (*Olaleye et al.*, 2018).

Attempt has been made in this particular research to apply some pre-harvest weed control system such as the use of pre-emergence and inter-row spacing so as to reduce post-harvest losses. Therefore, the objective of this study is to determine the effects of the different weed control methods on the physiochemical properties of cowpea flour, proximate composition, physical properties, and sensory qualities of cowpea-based pudding produced from cowpea flour.

2. Materials and methods

The Ife Brown variety of cowpea with three treatments was obtained from the Institute of Food Security, Environmental Resources and Agricultural Research (IFSERAR), Federal University of Agriculture Abeokuta (FUNAAB), Nigeria, while other equipment, such as cabinet dryer, sieve, or blender, was obtained from the Food Processing Laboratory, Department of Food Science and Technology, FUNAAB, Nigeria.

2.1 Treatment of cowpea grains

The cowpea grains obtained were grown with an inter-row spacing of 50 x 75 cm, and weeding was carried out as follows:

– Sample A – supplementary hoe weeding at 6 weeks after sowing (WAS);
– Sample B – two hoe weeding at 3 and 6 weeks after sowing (WAS);
– Sample C – three hoe weeding at 3, 6, and 9 weeks after sowing (WAS).

Preparation of cowpea flour

The cowpea flour was prepared in accordance with the method described by *Idowu et al.* (2017). The cowpea seeds were separated from their husks, dried, weighed, and stored during their processing into flour. Then they were cleaned, soaked, and decorticated. The detached hulls were decanted from the beans. The decorticated cowpea was dried at a temperature of 65°C in a hot air oven (GALLENKAMP SG3-08-169, UK) to a moisture content of 4.0% and dry-milled into flour using laboratory hammer mill (Fritsch, D-55743, Idar-oberstein, Germany). The milled sample was sieved using a 250-µm screen to obtain the flour.

2.2 Physicochemical properties of cowpea flour

2.2.1 Determination of amylose

The amylose content of cowpea flour was determined based on the iodine colorimetric method, as described by *Addy et al.* (2014). About 0.1 g of the starch sample was solubilized with 1 ml of 95% ethanol and 9 ml of 1 M NaOH, and heated in a boiling water bath for 10 min; 1 ml of the extract was made up to 10 ml with distilled water. To 0.5 ml of the diluted extract, 0.1 ml of acetic acid and 0.2 ml iodine solution (0.2 g I_2 + 2.0 g KI in 100 ml of distilled water) were added to develop a dark blue colour. The coloured solution was made up to 10 ml with distilled water and allowed to stand for 20 min for complete colour development. The solution was vortexed, and its absorbance was read on a spectrophotometer (Milton Spectronic 601, USA) at 620 nm. The absorbance of standard amylose with known amylose concentration was used to estimate the amylose content as follows:

$$\% \text{ Amylose } = \frac{\% \text{ amylose of standard x Absorbance of sample}}{\text{Absorbance of standard}} \quad (1)$$

2.2.2 Determination of titratable acidity

The method described by AOAC (2012) was used. Twenty-five millimetre of filtrate was transferred into a 125-ml conical flask, a 25-ml burette was filled with 0.1 N NaOH solution adjusted to zero, and then two to three drops of phenolphthalein indicator was added to the conical flask containing the filtrate. The resulting filtrate was then titrated with 0.1 N NaOH until there was a change in colour, and the volume was recorded. Titratable acidity and the volume were recorded. Titratable acidity was calculated as % citric acid:

$$\% \text{ acid} = \frac{\text{N x V x M}}{\text{S x 10}}, \quad (2)$$

where: N = normality of titrant – usually, 0.1 N NaOH solution is used (mEq/ml); V = volume of titrant (0.1 N NaOH solution) used (ml); M = molecular weight of the predominant acid in the sample divided by the number of hydrogen ions in the acid molecule that are titrated; S = mass of sample (g).

2.2.3 pH determination

The method described by AACC (2000) was used for the determination of pH. Dissolving 30 g of the cowpea flour blends with 90 ml of distilled water, the pH electrode (Thermo Russel RL150, Boston, USA) was placed in the filtrate after

being washed with distilled water, and the electrode was allowed to stabilize for a few moments. The pH value of the filtrate was taken after then.

2.2.4 Water absorption capacity

The method described by *Ojinnaka et al.* (2013) was used to determine the water absorption capacity. After weighting 1 g of cowpea flour into a centrifuge tube and mixing it with 10 ml of distilled water, the mixture was left to stand at room temperature for 30 minutes. Thereafter, it was centrifuged (GALLENKAMP Centrifuge Model 90 - 1, USA) at 3,500 rpm for 30 min. The clear supernatant was decanted and discarded. The adhering drops of water in the centrifuge tube were cleaned off with cotton wool, and the tube was weighed. Percentage water absorption capacity was calculated.

2.3 Preparation of cowpea-based pudding from cowpea flour

The method described by *Olaleye et al.* (2018) was used to prepare the cowpea-based pudding. Cowpea flour was poured into a mixing bowl, and 100 ml of warm water was added and mixed thoroughly to form a fluffy paste. It was then scooped into a small bowl with cover, packaged and steam cooked for about 40 min. The samples were then left for cooling for further analysis.

2.4 Proximate composition of cowpea-based pudding

Moisture content, total ash, crude fat, crude fibre, and crude protein were determined using standard methods, as described by AOAC (2012), while total carbohydrate was calculated by difference.

Colour properties of cowpea-based pudding

The method described by *Feili et al.* (2013) and *Oke et al.* (2017) was used. To measure the colour properties of the samples, Minolta Chroma Meter (CR-410, Japan) was used based on (CIE) L* a* b* scale after calibrating the instrument by covering a zero-calibration mask followed by a white calibration plate. Samples were analysed by placing them on a Petri dish, and then the images were captured on the samples. The colour attributes, such as lightness (L*), redness (a*), and yellowness (b*), were recorded.

2.5 Sensory acceptability of cowpea-based pudding

The method described by *Iwe* (2000) was used for the sensory acceptability of cowpea-based pudding. A laboratory-scale sensory acceptability test was

conducted, where 30 untrained panellists determined their preference on a nine-point hedonic scale (1 = dislike extremely and 9 = like extremely) in terms of texture, sweetness, crispness, oiliness, and overall acceptance among consumers.

2.6 Statistical analysis

Mean values of triplicate determinations of all the analyses were subjected to one-way analysis of variance (ANOVA) to determine significant differences, and the means were separated using Duncan's multiple-range test at $p < 0.05$.

3. Results and discussions

3.1 Physiochemical composition of cowpea flour

The results for the physicochemical composition of cowpea flour are presented in *Table 1*. The pH values ranged from 4.85 to 5.10. The pH values of all samples were in acidic range, and total titratable acidity (TTA) decreased from 0.05% to 0.02%. The pH value and total titratable acidity showed that acidity decreased as the frequency of hoe weeding increased. Low total titratable acidity of the samples is a proper indication of the good absorption of mineral elements and also an indicator that the product will have a long shelf life because the presence of acidity prevents and/or delays the growth of spoilage microbes (*Abioye et al.*, 2011; *Otunola & Afolayan*, 2018).

There were also significant ($p < 0.05$) differences in water absorption capacity (WAC) and amylose content. The result for WAC values showed a ranged from 276% to 287%. WAC is a very important property in food formulations required for bulking, consistency and in baking applications with an effect on the final product characteristics such as flavour retention, mouthfeel, and shelf life (*Ikegwu et al.*, 2010). The increase in the frequency of hoe weeding led to increase in the capacity of the flour to absorb water by a decrease in values.

The amylose content of the cowpea flours ranged from 22.04% to 24.60%. A higher amylose content has been reported by Kim et al. (2018) in the starch of cowpea and mung bean, in the range of 36%–39%, but cowpea results showed a lower amylose content; this may be due to the cowpea variety type. The amylose content affects the physicochemical properties of starch such as viscosity, swelling power, gelatinization capacity, retrogradation, and starch crystallinity (*Zhenghong et al.*, 2003; *Riley et al.*, 2014).

Table 1. Physicochemical composition of cowpea flour

Samples	pH	TTA (%)	WAC (%)	AMYLOSE (%)
A	5.10 ± 0.14^{b}	0.05 ± 0.01^{ab}	281.40 ± 0.23^{b}	24.60 ± 0.02^{c}
B	4.85 ± 0.07^{a}	0.05 ± 0.01^{ab}	287.09 ± 0.98^{b}	22.85 ± 0.04^{b}
C	5.00 ± 0.00^{ab}	0.02 ± 0.01^{a}	276.00 ± 0.02^{ab}	22.04 ± 0.03^{a}

Notes: Mean values with different superscripts within the same column are significantly different ($p < 0.05$). TTA: total titratable acidity; WAC: water absorption capacity; A – supplementary hoe weeding at 6 WAS; B – two hoe weeding at 3 and 6 WAS; C – three hoe weeding at 3, 6, and 9 WAS.

3.2 Proximate composition of cowpea-based pudding produced from cowpea flour

The proximate composition results of the cowpea-based pudding produced from cowpea flour are presented in *Table 2*. Moisture content results showed an increase in value from 74.26% to 76.15%. The results showed that sample C had the highest value (76.15%) of moisture content and was significantly different ($p < 0.05$) from sample B and sample A, which recorded 75.89% and 74.26% respectively. Sample A had the lowest moisture content when compared to samples B and C, and this is an indicator of good storage stability and longer shelf life. The moisture content obtained in this study was close to the range of 64.90%–69.70% reported by *Bamgboye & Adepoju* (2015). The differences in the moisture content could be attributed to differences between the various bean cultivars and their processing time.

Table 2. Proximate composition of cowpea-based pudding produced from cowpea flour

Samples	Moisture content (%)	Crude fat (%)	Total ash (%)	Crude fibre (%)	Crude protein (%)	Total carbohydrate (%)
A	74.26 ± 0.05^{b}	0.71 ± 0.02^{b}	0.94 ± 0.01^{c}	0.75 ± 0.01^{b}	17.83 ± 0.02^{c}	5.55 ± 0.02^{b}
B	75.89 ± 0.05^{c}	0.63 ± 0.00^{a}	0.79 ± 0.01^{b}	0.69 ± 0.01^{a}	16.88 ± 0.06^{b}	5.15 ± 0.02^{a}
C	76.15 ± 0.04^{d}	0.64 ± 0.01^{a}	0.75 ± 0.00^{a}	0.66 ± 0.01^{a}	16.70 ± 0.01^{a}	5.11 ± 0.01^{a}

Notes: Mean values with different superscripts within the same column are significantly different ($p < 0.05$). A – supplementary hoe weeding at 6 WAS; B – two hoe weeding at 3 and 6 WAS; C – three hoe weeding at 3, 6, and 9 WAS.

Results on crude fat showed that sample A had the highest value (0.71%), which was significantly different ($p < 0.05$) from other samples. Sample B had the lowest value (0.63%) of crude fat. The lower fat content of sample B reduces the rate at which rancidity sets in, thereby increasing its shelf life.

Total ash value ranged from 0.75% to 0.94%. Total ash content is an indication of the amount of minerals present in the cowpea-based pudding, which contributes to a specific number of positive functions in the body (*Baah et al.*, 2009). The total ash value of sample C obtained in this study is an indication that sample C is not a good source of mineral.

The high value of crude fibre recorded by sample A (0.75%) is advantageous, as fibre is important in food because it absorbs water and provides roughage for the bowels, aids digestion, lowers the plasma cholesterol level in the body, and prevents several diseases such as irritable colon, cancer, and diabetes (*Elleuch et al.*, 2011; *Idowu et al.*, 2017). The low crude fibre and low protein in sample C is an indicator that the puddings are low-calorie foods that may be very useful in weight management. There were significant differences ($p < 0.05$) in the crude protein content of cowpea-based pudding, that of sample A having the highest (17.85%) and sample C the lowest (16.70%).

The high crude protein observed in sample A (17.83%) could be attributed to the significant quantity of protein (about 24%) in cowpea seed (*Jimoh & Olatidoye*, 2009). The crude protein obtained in this study was higher than the values of 18.80%–21.20% and 16.50%–21.87% reported by *Bamgboye & Adepoju* (2015) and *Olaleye et al.* (2018). This could be due to the differences in the types of legumes used in this study.

The total carbohydrate value ranged from 5.11% to 5.55%, sample A having the highest value. This implies that sample A will provide more glucose to the body when consumed as carbohydrates, which are known to supply glucose to the body, which is then converted to energy used to support various bodily functions and physical activity. Sample A exhibits a higher total ash, crude fibre, and total carbohydrate content when compared with sample B and sample C. Total ash, crude fibre, and carbohydrate content decreases with the increasing frequency of hoe weeding, which means that the nutritional content is low.

3.3 Colour properties of cowpea-based pudding produced from cowpea flour

The colour properties of cowpea-based pudding produced from cowpea flour are presented in *Table 3*. Lightness values ranged from 27.92 to 29.02 while redness values from 7.89 to 9.68. This could be as a result of the process of planting conditions and a significant change in the colour of the seed during the process

of soaking and dehulling, while the removal of the seed coat also had a great effect on the colour of the cowpea-based pudding. Sample B recorded the highest yellowness value of 14.11, while sample C recorded the lowest yellowness value of 10.81. The recorded differences in colour properties may be due to the presence of several types of colour constituents (phenolic compounds) present in the cowpea seed (*Sombié et al.*, 2018) used in this study.

Table 3. Colour properties of cowpea-based pudding produced from cowpea flour

Samples	L*	a*	b*
A	29.02 ± 0.03^{c}	9.18 ± 0.04^{b}	13.74 ± 0.08^{b}
B	28.55 ± 0.07^{b}	9.68 ± 0.09^{c}	14.11 ± 0.25^{c}
C	27.92 ± 0.03^{a}	7.89 ± 0.09^{a}	10.81 ± 0.11^{a}

Notes: Mean values with different superscripts within the same column are significantly different ($p < 0.05$). A – supplementary hoe weeding at 6 WAS; B – two hoe weeding at 3 and 6 WAS; C – three hoe weeding at 3, 6, and 9 WAS; L* – lightness; a* – redness; b* – yellowness.

3.4 Sensory score of cowpea-based pudding produced from cowpea flour

Results of the sensory score of cowpea-based pudding are shown in *Table 4*. Significant ($p > 0.05$) differences were not observed in all of the sensory attributes. The texture and the taste ranged from 5.83 to 6.09 and from 5.06 to 6.19 respectively. Cowpea-based pudding produced from sample A had the highest score for texture and taste, while cowpea-based pudding produced from sample C had the lowest score. The colour and appearance ranged from 5.26 to 5.90 and from 5.13 to 5.58 respectively. The low score obtained for the colour and appearance of the cowpea-based pudding could be attributed to the different colour of the cowpea grains. However, the cowpea-based pudding from sample A had the highest score for colour and appearance, while sample B had the lowest score. The aroma ranged between 5.13 and 5.81. The overall acceptability expresses how the consumers or panellists accept the product generally. The overall acceptability ranged from 5.90 to 6.47. It was observed that cowpea-based pudding from sample A and C had the highest overall acceptability, which could be due to the familiarity of the panellist with cowpea-based pudding prepared from cowpea flour.

Table 4. Sensory score of cowpea-based pudding produced from cowpea flour

Samples	Texture	Taste	Colour	Appearance	Aroma	Overall acceptability
A	5.58 ± 1.73^{a}	5.06 ± 1.53^{a}	5.26 ± 1.69^{a}	5.13 ± 1.57^{a}	5.81 ± 1.38^{a}	6.42 ± 1.26^{a}
B	6.03 ± 1.54^{a}	5.55 ± 1.39^{ab}	5.90 ± 1.54^{a}	5.58 ± 1.46^{a}	5.13 ± 1.34^{a}	5.90 ± 1.11^{a}
C	6.09 ± 1.59^{a}	6.19 ± 1.45^{b}	5.84 ± 1.48^{a}	5.50 ± 1.16^{a}	5.69 ± 1.47^{a}	6.47 ± 1.41^{a}

Notes: Mean values with different superscripts within the same column are significantly different ($p < 0.05$). A – supplementary hoe weeding at 6 WAS; B – two hoe weeding at 3 and 6 WAS; C – three hoe weeding at 3, 6, and 9 WAS.

4. Conclusions

The study revealed that the effect of different weed control methods employed significantly affected the performance of cowpea. The physicochemical and proximate composition of the cowpea flour and cowpea-based pudding, respectively, decreased as the frequency of weeding increased. The sensory scores show that cowpea-based pudding produced from cowpea flour with supplementary hoe weeding at 6 WAS and three hoe weeding at 3, 6, and 9 WAS was accepted by the panellist to a lesser degree.

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Nutritional quality and health benefits of roselle calyces

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Abstract. Roselle calyces (*Hibiscus sabdariffa* L.) were evaluated through a critical study of existing research works on health benefits, mineral compositions, bioactive compositions, mechanisms, and possible research gaps. The use of roselle calyces as an alternative to synthetic food dyes, addressing growing global challenges of overweight, obesity, and cardiovascular diseases, was evaluated and encouraged. Studies indicate the attenuation of obesity by chlorogenic acid (the predominant phenolic compound in roselle calyx) via mechanisms associated with the UCP-1 and PGC-1α pathways, resulting in reduced blood lipid levels, reduced fat accumulation in the liver, and increased thermogenesis through fat metabolism. Minimum inhibitory concentration (MIC) of known bacteria and fungi, such as *Listeria monocytogenes, Escherichia coli, Bacillus cereus, Salmonella typhimurium*, *Candida tropicalis*, and *Candida krusei*, were studied. More research, however, needs to be conducted on organic acids present in roselle calyces to look into their possible applications and maximize their possible benefits.

Keywords and phrases: oxidative stress, mechanism, weight control, antimicrobial, anthocyanin

1. Introduction

Grown predominantly for its calyx, *Hibiscus sabdariffa* L. belongs to the Malvaceae family and is widely distributed around the subtropics and the tropical hemisphere due to its ability to thrive on a relatively wide range of soil conditions (*Riaz et al.*, 2021). It is a good source of ascorbic acid, anthocyanins (mostly delphinidin-3-sambubioside and cyanidin-3-sambubioside), organic acids, phenolic compounds, as well as mineral constituents such as calcium, iron,

magnesium, and potassium. Hence, it could prove effective in treating ailments resulting from mineral deficiencies such as hypocupremia, hypomagnesemia, and anaemia (*Pham et al.*, 2014; *Shruthi & Ramachandra*, 2019).

Roselle calyx possesses antioxidant, anti-hypertensive, anti-microbial, anti-proliferative, and anti-hyperglycaemic properties (*Banwo et al.*, 2022; *Puro et al.*, 2017). Its suitability in beverage, colorant, and wine production (*Alobo & Offonry*, 2009; *Reddy et al.*, 2022) has significantly contributed to increased demand for roselle globally.

Roselle extract is often seen as a drink for the economically disadvantaged, resulting in disparity between a number of consumers in rural and urban areas, particularly in developing countries, prompting population perspective of the health benefits of roselle calyx and its extract as inflated or exaggerated.

This review was designed with the aim of evaluating existing research findings on the nutritional quality, antimicrobial potency, and health benefits of roselle calyx.

2. Methodology

This review was carried out without restriction to the year of article publication. Keywords relevant to this topic were used to search for relevant articles on academic research sources such as Google Scholar, ResearchGate, PubMed, and ScienceDirect. A holistic approach was taken to carefully evaluate research findings, identify research gaps, and provide valuable suggestions.

3. Results and discussion

Nutritional and bioactive composition of roselle calyces

Data obtained on the mineral and proximate composition of roselle calyces based on multiple research findings, as shown in *tables 1–2*, highlight their nutritional benefits. 100 grams of roselle calyx would sufficiently satisfy the recommended dietary allowance (RDA) for calcium, iron, manganese, and copper.

A high concentration of iron in roselle calyces indicates that it can be used in the treatment of anaemia, a condition in which the blood lacks sufficient haemoglobin to transport oxygen to parts of the body where it is needed, resulting in reduced physical capabilities and cognitive decline in humans (*Camaschella*, 2019; *Clark*, 2008). Low crude lipids (0.46–2.01%), high protein content (4.71–8.31%), carbohydrates (68.75–69.62%), and crude fibre (4.68–11.53%) were observed in roselle calyx, the latter of which helps to ease bowel movement, keep the digestive system clean, prevent overfeeding, and flush out carcinogens (*Barber et al.*, 2020).

Table 1. Mineral composition of roselle calyx

Elements	Concentration (mg/100g)	RDA (mg)		Reference
		Male	Female	
Calcium (Ca)	1,583	1000	1000	*Babalola et al.,* 2001
	2,105.78			*Riaz et al.,* 2021
	912.15			*Abou-Arab et al.*, 2011
Potassium (K)	2,060	4700	4700	*Babalola et al.*, 2001
	1,263			*Riaz et al.*, 2021
	20.60			*Abou-Arab et al.*, 2011
Magnesium (Mg)	316	400	310	*Babalola et al.,* 2001
	280.12			*Riaz et al.*, 2021
	315.21			*Abou-Arab et al.*, 2011
Sodium (Na)	5.50	1500	1500	*Babalola et al.,* 2001
	7.74			*Riaz et al.*, 2021
	6.62			*Abou-Arab et al.*, 2011
Iron (Fe)	37.80	8	18	*Babalola et al.,* 2001
	21.11			*Riaz et al.*, 2021
	37.80			*Abou-Arab et al.*, 2011
Zinc (Zn)	6.5	11	8	*Babalola et al.,* 2001
	5.73			*Riaz et al.*, 2021
	6.51			*Abou-Arab et al.*, 2011
Manganese (Mn)	2.24	2.3	1.8	*Riaz et al.*, 2021
	2.39			*Abou-Arab et al.*, 2011
Copper (Cu)	3.68	0.9	0.9	*Riaz et al.*, 2021
	4.32			*Abou-Arab et al.*, 2011

Note: RDA = recommended dietary allowance for adults.

Table 2. Proximate composition of roselle calyx

	Value (%)	Reference
Moisture	10.50	*Balarabe*, 2019
	7.60	*Adanlawo & Ajibade*, 2006
	9.22	*Cid-Ortega & Guerrero-Beltran*, 2014
Ash	5.69	*Puro et al.*, 2017
	11.67	*Balarabe*, 2019
	12.24	*Adanlawo & Ajibade*, 2006
Proteins	4.10	*Balarabe*, 2019
	7.51	*Abou-Arab et al.*, 2011
	4.71	*Adanlawo & Ajibade*, 2006
Carbohydrate	68.75	*Adanlawo & Ajibade*, 2006
	69.62	*Abou-Arab et al.,* 2011
Crude fibre	11.53	*Puro et al.*, 2017
	11.17	*Abou-Arab et al.,* 2011
	4.69	*Adanlawo & Ajibade*, 2006
Crude lipid	1.0	*Balarabe*, 2019
	2.01	*Adanlawo & Ajibade*, 2006
	0.46	*Abou-Arab et al.,* 2011

While the nutraceutical and therapeutic benefits of roselle calyces have largely been attributed to their high anthocyanin concentration (*Table 3*), several studies have proven that this is not completely true. Some research has attributed health benefits such as weight loss and blood pressure regulation to the mineral content, proximate composition, and presence of beneficial organic acids in the calyces of roselle (*Morales-Luna et al.*, 2019).

Roselle calyx was found to contain phenolic compounds such as catechin, caffeic acid, rutin, gallic acid, cinamic acid, chlorogenic acid, and benzoic acid, some of which have been proven to possess anti-inflammatory, anti-hypertensive, hypocholesterolemic, anti-diabetic, anti-hyperglycemic, and anti-microbial properties (*Banwo et al.*, 2022). Research conducted by *Olthof et al.* (2001) on 7 subjects indicates 33% and 95% absorption of chlorogenic acid and caffeic acid, respectively, consumed by the human body.

Anthocyanins in roselle calyx

The characteristic reddish/purplish coloration of roselle calyx exists due to the presence of anthocyanins such as delphinidin-3-sambubioside, cyanidine-3-sambubioside, delphinidin-3-glucoside, and cyanidine-3-glucoside (*Wu et al.*, 2018).

Table 3. Concentration of ascorbic acid, anthocyanin, and total phenolic content of roselle calyx

	Unit	Value	Reference
Total phenolic	mg GAE/g	37.42	*Abou-Arab et al.,* 2011
	mg GAE/g	38.58	*Riaz et al.*, 2021
	mg GAE/g	41.07	*Sirag et al.*, 2014
Anthocyanin	mg/100g	635.86	*Riaz et al.*, 2021
	mg/100g	565	*Abdel-Moemin*, 2016
	mg/100g	80.1	*Puro et al.*, 2017
Ascorbic acid	mg/100g	63.5	*Babalola et al.,* 2001
	mg/100g	140.13	*Abou-Arab et al.,* 2011

This compound can be harnessed as an alternative to synthetic food dyes, the latter of which may pose negative effects such as hyperactivity, allergies, asthmatic reactions, and possibly carcinogenic effects in humans (*Abdel-Moemin*, 2016).

Research conducted by *Hernández-Nava et al.* (2023) and *Abdel-Moemin* (2016) on the use of roselle calyx in the production of biscuits and cupcakes, respectively, reported on colour difference resulting from the incorporation of roselle calyx. Both products obtained good sensory evaluation and were proven to have significantly improved the anthocyanin content and antioxidant quality of the products as compared to the respective control samples, thereby making for a healthier diet. Anthocyanin at a concentration of 3 mg/mL exhibited cytotoxicity towards leukaemia HL-60 cells in a dose- and time-dependent manner through the activation of c-Jun and P38 MAP kinases, triggering Bcl-2 activation, thereby resulting in the induced apoptosis of HL-60 cells (*Chang et al.*, 2005).

Antimicrobial properties of roselle calyx

Antimicrobial resistance has increasingly become a threat to development, global health, and food security. Reduced effectiveness of antibiotics on infections ranging from mild to life-threatening occurs naturally or from the inappropriate use of antibiotics, resulting in increased medical costs and mortality rates (*WHO*, 2020). This mounting challenge has prompted researchers to explore alternative pathways for combating bacterial infections.

Microbes exist in our environment under various conditions and can contaminate foods at any stage of production, resulting in reduced shelf life of agricultural produce and, in some cases, can have adverse effects on the health of consumers (*Gonelimali et al.*, 2018). Research findings, as indicated in *Table 4*, highlight the

inhibitory ability of roselle calyx extract against bacteria associated with food contamination and spoilage such as *B. cereus, E. coli, S. typhimurium,* and *P. aeruginosa*.

Table 4. Minimum inhibitory concentration (MIC) of roselle calyx extract against bacteria and fungi

	Species	Calyx extract	MIC	Reference
Bacteria	*S. aureus*	Aqueous	2.342 mg/mL	*Hamrita et al.*, 2022
		Methanol	2.342 mg/mL	*Hamrita et al.*, 2022
		Aqueous	112 µg/mL	*Chao & Yin*, 2009
		Ethanol	72 µg/mL	*Chao & Yin*, 2009
	L. monocytogenes	Aqueous	136 µg/mL	*Chao & Yin*, 2009
		Ethanol	84 µg/mL	*Chao & Yin*, 2009
	P. aeruginosa	Aqueous	9.375 mg/mL	*Hamrita et al.*, 2022
		Methanol	2.342 mg/mL	*Hamrita et al.*, 2022
	E. coli	Aqueous	128 µg/mL	*Chao & Yin*, 2009
		Ethanol	72 µg/mL	*Chao & Yin*, 2009
	B. cereus	Aqueous	144 µg/mL	*Chao & Yin*, 2009
		Ethanol	96 µg/mL	*Chao & Yin*, 2009
	S. typhimurium	Aqueous	120 µg/mL	*Chao & Yin*, 2009
		Ethanol	80 µg/mL	*Chao & Yin*, 2009
Fungi	*Candida tropicalis*	Aqueous	9.375 mg/mL	*Hamrita et al.*, 2022
		Methanol	9.375 mg/mL	*Hamrita et al.*, 2022
	Candida krusei	Aqueous	9.375 mg/mL	*Hamrita et al.*, 2022
		Methanol	9.375 mg/mL	*Hamrita et al.*, 2022

Note: MIC = minimum inhibitory concentration.

Anti-hypertensive properties of roselle calyx

Hypertension has greatly affected morbidity and mortality rates globally, with a prevalence of 20–30% observed in developing countries, especially among the older generation (*Holm et al.*, 2006).

A study by *Herrera-Arellano et al.* (2004) on the use of roselle calyx as a remedy for hypertension indicated a significant decrease in systolic (139.05 to 123.73 mmHg) and diastolic (90.81 to 79.52 mmHg) blood pressure in hypertensive test subjects (30–80 years) orally administered 10 g/0.5L of Hibiscus sabdariffa extract

daily as treatment for a period of four (4) weeks after abstaining from other forms of hypertensive treatment four (4) weeks prior to the study.

Aliyu et al. (2014) obtained similar results: orally administered 15 mg/kg of Hibiscus sabdariffa extract effected the attenuation of the sympathetic nervous system. Anti-hypertensive properties exhibited by roselle calyx can also be attributed to mechanisms associated with the inhibition of angiotensin-converting enzymes (ACE) by anthocyanin, resulting in reduced serum sodium concentration without compromising potassium levels in a dose-dependent manner (*Ojeda et al.*, 2010). Anthocyanin prevents free radical oxidation by donating protons, which aids in the regeneration of acyl-glycerol molecules (*Reis et al.*, 2016), thus reducing the risk of hypertension by preventing damage to the endothelium responsible for maintaining balance between vasoconstriction and vasodilatation (*Grossman*, 2008).

Weight control

Overweight and obesity have been concomitant with a series of health issues ranging from diabetes, musculoskeletal disorders, cardiovascular diseases, and some cases of cancer. According to the WHO, 39% of the world's population 18 years of age and older in 2016 (1.9 billion people) were overweight, among which 650 million were obese. The obese or overweight population of adolescents and children in the same year (2016) within the age range of 5 and 19 years was 340 million (*WHO*, 2021).

The disturbing increase in cases of overweight and obesity could in large part be attributed to the consumption of high-calorie diets and physical inactivity, prompting the need for low-calorie diets. The inhibitory activity of roselle calyx extract against porcine pancreatic α-amylase (PPA) and ATP-citrate lyase, as observed by *Hansawasdi et al.* (2000), indicates that the consumption of roselle calyx could prove to be an effective method of reducing glucose absorption in the body.

Studies indicate that obesity can be attenuated by chlorogenic acid (the predominant phenolic compound in roselle calyx) via mechanisms associated with the UCP-1 and PGC-1α pathways, resulting in reduced blood lipid levels, reduced fat accumulation in the liver, and increased thermogenesis through fat metabolism (*Zhong et al.*, 2020).

Antioxidant activities of roselle calyx

Antioxidants are vital to humans, as they are known to prevent the oxidation of body metabolites by removing reactive oxygen species (*Brantley & Sternberg*, 2012). Reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as singlet oxygen ($^1O_2$), superoxide radicals (O_2^-), hydroxyl radicals (OH^-), hydrogen

peroxide (H_2O_2), nitric oxide (NO^-), and nitrogen dioxide (NO_2^-) in the body, could lead to the quick build-up of oxidative stress, a phenomenon resulting from an imbalance of production and accumulation of ROS and RNS in cells and tissues (*Pizzino et al.*, 2017).

Accumulation of free radicals in the body could result in health problems such as cancer, inflammatory and cardiovascular diseases, cataract and neurodegenerative diseases, and brain aging (*Lobo et al.*, 2010).

4. Conclusions

Consumption of roselle calyces is encouraged, as they provide a good percentage of the recommended daily mineral intake and have proven to be a cost-effective means of addressing lots of health-related issues. This review highlights the accomplishment of in-vivo studies on the anti-hypertensive effectiveness of roselle calyx extract. However, studies on the apoptosis of cancer cells by Hibiscus sabdariffa extract have been limited to in-vitro analysis; there is a need to scientifically establish the effectiveness of roselle extract on cancer patients. Few detailed studies exist on the potential application of organic acids present in roselle calyx and their possible role in addressing health-related issues.

Roselle calyx extract inhibitory effect against *S. aureus*, *L. monocytogenes*, *P. aeruginosa*, *E. coli*, *B. cereus*, *S. typhimurium*, *Candida tropicalis*, and *Candida krusei* in time- and dose-dependent manner highlights the need for more research to be conducted on the incorporation of roselle calyces as a cost-effective method for improving the shelf life of food products as compared to controls.

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Mineral and anti-nutritional properties of pearl millet and pumpkin leaf flour as affected by fermentation

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Abstract. This study was aimed at determining the mineral and anti-nutritional properties of naturally fermented millet and pumpkin leaf flour blends. The millet grains were allowed to ferment spontaneously for 24 hrs and 48 hrs and were processed into flour. Dried pumpkin leaves were blended into flour and substituted using D-optimal mixture design, which resulted in ten experimental runs. The mineral content and the anti-nutritional properties of the flour blend formulation were analysed. Duncan's multiple range test was used to evaluate the mean at $p < 0.05$ with SPSS version 21.0. Significant differences were observed in the mineral and anti-nutritional composition of the fermented millet and pumpkin leaf flour blends at 24 hrs and 48 hrs of fermentation time respectively. Calcium, potassium, and iron content increased significantly ($p < 0.05$) with increasing the amount of pumpkin leaf flour in the flour blends. The values for tannins and total phenolic composition ranged from 0.089 to 0.162% and from 0.075 to 0.120% for 24 hrs and from 0.080 to 0.141% and from 0.060 to 0.120% for 48 hrs of fermentation time respectively. Results showed that fermentation technique could be used to enrich the nutritional and bioactive potential of millet.

Keywords and phrases: tannin, phytate, trypsin inhibitor, phenol, D-optimal mixture

1. Introduction

Millet is a seeded grass that is widely grown for human and animal nutrition all over the world (*Ranasalva & Visvanathan*, 2014). It is an important crop in semi-arid tropics of Africa and Asia, particularly in Nigeria, due to its ability to grow under adverse weather conditions such as little rainfall, no fertilizer availability, or various other scarcities; therefore, they are mostly recommended for farmers having difficult circumstances (*Soumya et al.*, 2016). The most common types of cultivated millet are foxtail millet (*Setaria itallica*), finger millet (*Eleusine coracona*), pearl millet (*Pennisetum typhoideum*), and proso millet (*Panicum miliaceum*) (*Thilagavathi et al.*, 2015). Pearl millet provides higher energy compared to other cereal grains such as rice and wheat and is considered a significant source of nutrients such as calcium, potassium, thiamine, niacin, and riboflavin (*Shweta*, 2015). Millet can be used in producing food and beverage products such as papad, muruku, bread, hot kolukattai, milk, malt beverage, and alcoholic beverage (*Singh et al.*, 2021; *Mahajan et al.*, 2021). Due to the presence of tannins, polyphenolics, and phytic acid, the bioavailability of these nutrients is low. They are typically considered anti-nutrients and have been associated with inhibitory effect on protein and starch digestibility and mineral bioavailability. Studies have shown that these phytochemicals can be reduced substantially by food processing operations such as dehulling, malting, fermentation, and heat treatment (*Marston et al.*, 2016).

Fermentation is one of the oldest and widely used methods for processing millet, especially in Africa and other developing countries where modern food preservation methods are rarely available. It is a process involving the transformation of substrates (millets) into new products through the action of certain microorganisms (lactic acid bacteria and yeasts). This is regarded as an economical traditional processing technique adopted to yield large amounts of products. During fermentation, enzymes are activated through the action of microorganisms leading to changes in the pH; this is in addition to other biochemical changes that occur during this process leading to the modification of the substrate (*Adebiyi et al.*, 2018; *Srivastava et al.*, 2020). These biochemical changes contribute to preservative properties, improved flavours, and significant increase in nutritional properties (*Obilana et al.*, 2014). Fermentation also ensures the safety of the food by suppressing the growth and survival of undesirable microflora. Fermentation has been reported to effectively improve the nutritional quality of millet by increasing protein content, in-vitro protein digestibility (IVPD), and mineral extractability (*Ranasalva & Visvanathan*, 2014; *Adebiyi et al.*, 2018). Other significant roles of fermentation in millet processing include the development of a wide variety of flavours, aromas, and textures, detoxification, and a decrease in cooking time (*Jay et al.*, 2005; *Adebiyi et al.*, 2018).

Other sources of nutrients, such as legumes and leaves, could be used to supplement cereal flours in complementary porridge and bakery products to

improve the nutritional and sensory qualities of such products (*Chikondi et al.*, 2018). The consumption of vegetables, such as pumpkin leaves, has a significant impact on human health: they typically protect against chronic diseases and contain large amounts of iron, folic acid, vitamin A and C. It is a drought-tolerant plant; the young shoots and leaves are used in cooking soups, yam and vegetable sauces and also for medicinal purposes (*Mashiane et al.*, 2021). Deficiency of iron (anaemia) is a major problem in underdeveloped and developing countries (*Llanos et al.*, 2016). The causes of anaemia can be classified into blood loss, reduced production and increased destruction of red blood cells (*Thilagavathi et al.*, 2015). *Debasmita* and *Binata* (2017) reported that the major cause of anaemia is the inadequate nutrient intake of iron, vitamins A, B_{12}, C, folic acid, niacin and pantothenic acid, which are also responsible for maintaining the level of haemoglobin in the blood. To prevent the growing rate of anaemia, dietary improvement, fortification, and supplementation are beneficial ways for the entire population or for certain groups of people. The nutritional potentials of millet and fluted pumpkin leaves as composite flour in food products would enhance food security and improve the overall health status of consumers of such formulated food products (*Adeyeye*, 2016). Many researchers (*Onuoha et al.*, 2017; *Srivastava et al.*, 2020; *Devi & Rajendran*, 2021; *Azeez et al.*, 2022) have worked on the fermentation of pearl millet with other legumes, however, not in combination with pumpkin leaves. Therefore, the objective of this work is to determine the effect of fermentation on the minerals and anti-nutritional properties of flours from fermented pearl millet and fluted pumpkin leaves.

2. Materials and methods

Millet grains and fluted pumpkin leaves were procured in 2022 at the Odo-Eran market, Abeokuta Ogun State, Nigeria.

Fermented millet flour production

Fermented millet flour was produced according to the method described by *Adebayo-Oyetoro et al.* (2017). The millet grains were cleaned and soaked in potable water for 24 hrs. They were wet milled and sieved through a fine-mesh sieve (muslin cloth). The millet slurry was allowed to ferment naturally in clean plastic bucket for 24 hrs and 48 hrs. The fermented meal was then pressed using a fine-mesh sieve to produce the fermented cake. The cake was dried using a cabinet drier (LEEC Limited, Serial No 3114, United Kingdom) at 60°C for 10 hrs and milled with a milling machine (Fritsch, D-55743, Idaroberstein-Germany) to produce the fermented millet flour and was packaged in polyethylene bags.

Fluted pumpkin leaf flour production

Fluted pumpkin leaf flour was produced using the modified method of *Lawal et al.* (2021). Pumpkin leaves were washed thoroughly with tap water to remove dirt and sorted to remove stalks. The green leaves were drained using a plastic sieve, then sliced and dried at 60°C for 6 hrs using cabinet drier (LEEC Limited, Serial No 3114, United Kingdom). Dried pumpkin leaves were milled using blender, cooled and stored in high-density polyethylene bags.

Formulation of fermented pearl millet and fluted pumpkin leaf flour blends

Using D-optimal design, different blends of composite flour samples were prepared by combining fermented pearl millet and fluted pumpkin leaf flour in the following ratios: 93.75:6.25, 91.25:8.75, 90.00:10.00, 90.00:10.00, 95.00:5.00, 92.50:7.50, 92.50:7.50, 90.00:10.00, 95.00:5.00, and 100.00:0.00.

Mineral analysis of fermented pearl millet and fluted pumpkin leaf flour blends

The mineral content of the samples was assessed using the procedure of *AOAC* (2000). Calcium, iron, sodium, and potassium were measured using atomic absorption spectrophotometer (Thermo scientific S Series Model GE 712354) after they have been digested with perchloric-nitric acid mixture. Prior to digestion, 0.50 g of samples were weighed into a 125-ml Erlenmeyer flask, over which 4 ml of concentrated perchloric-nitric acid, 25 ml of concentrated nitric acid, and 2 ml of concentrated sulphuric acid were added under a fume hood. The contents were mixed, heated gently in a digester (Buchi Digestion unit K-424) with low to medium heat on a hot plate under perchloric acid fume hood, and heating was continued for about 30 s until the appearance of dense white fume. Then it was allowed to cool followed by addition of 50 ml distilled water. The solution was allowed to cool and filtered completely with a wash bottle into a Pyrex volumetric flask and then made up with distilled water. The solution was then read on atomic absorption spectrophotometer.

Determination of tannins

The determination of tannin content was done using the method of *Swain* (1979). Ground sample (0.2 g) was measured into a beaker (50 ml), 20 ml of 50% methanol was added, covered, and placed in 80°C water bath for about 1 h and was stirred to avoid clumping. Double-layered Whatman No. 1 filter paper was used in the filtration of solution into a volumetric flask (100 ml), and 50% methanol was used for rinsing. It was made up to the mark with distilled water and mixed;

an extract of 1 ml was pipetted into a volumetric flask (50 ml); 20 ml of distilled water and 10 ml of 17% Na_2C0_3 and Folin–Denis reagent were thoroughly mixed. This was made up with distilled water and allowed to stand for about 20 min until a bluish-green colour was visible. Standard tannic acid solutions in the range of 0–10 (µg/L) were treated similarly to the 1 ml of the sample. The absorbance of tannic acid standard solutions as well as the sample were read on the Spectronic 21D Spectrophotometer at a wavelength of $\lambda = 760$ nm.

Determination of trypsin inhibitor activity

Determining trypsin inhibitor activity was done by the method described by *Kakade et al.* (1974). Flour sample of 1 g was extracted with 50 ml of 0.01 M of NaOH for 1 h, and the pH of the resulting slurry was adjusted to between 9.4 and 9.6 with 1 M NaOH or 1 M HCl. At this stage, the slurry was shaken and left at 4°C overnight; but occasionally it was more convenient to stir at ambient temperature for 3 hrs or to macerate continuously (Ultra-Turrax) for 2 min. After extraction, the suspension was shaken and diluted with water, so an amount of 1 ml produced trypsin inhibition of between 40% and 60%. Trypsin solution of 2 ml was added to the test tube; the tubes were placed in 37°C water bath and were allowed to stay there for 10 min. Then 1 ml of 30% acetic acid was added and the contents were thoroughly mixed and filtered with Whatman No. 3. The absorbance was measured at $\lambda = 410$ nm against a blank reagent.

Determination of total phenolic content

The method described by *Turturică et al.* (2016) was used in the determination of total phenolic content. 1 g of sample was ground with pestle and mortar with the addition of 10 ml 80% ethanol. The mixture was centrifuged at 10,000 rpm for 20 min. The supernatant was kept, and the residue was further extracted with 80% ethanol, centrifuged, and exposed for evaporation to take place. The residue was to dissolve in 5 ml of distilled water. The sample was measured into the test tubes, and the volume of each was made up with distilled water to 3 ml. 0.5 ml of Folin–Ciocâlteu reagent was added to the test tubes. After about 3 min, 2 ml sodium carbonate (20%) solution was added to each tube. The contents of the test tubes were mixed thoroughly, and the tubes were placed in a water bath for 1 min. It was allowed to cool, and absorbance was read at $\lambda = 650$ nm against a blank reagent.

Determination of phytate

The method described by *Maga* (1982) was used in the determination of phytate. 2 g of sample was soaked in 20 ml of 0.2 M of HCl for 3 hrs and filtered. The filtrate (0.5 ml) was mixed with 1 ml of 70% ferric-ammonium-sulphate solution in a test

tube, boiled for 30 min in the water bath (100°C), cooled in ice, and centrifuged at 3,000 rpm for 15 min. 1 ml of the supernatant was mixed thoroughly with 0.1 M of 2,2-pyridine solution (1.5 ml), and the absorbance was read at λ = 519 nm using spectrophotometer.

Statistical analysis

Each analysis was carried out in triplicate. Mean values of the three replicates' results were subjected to one-way analysis of variance (ANOVA) to determine the significant difference, and the means were separated using Duncan's multiple range test at 95% confidence level ($p < 0.05$).

3. Results and discussions

The mean values for the mineral composition of fermented millet grains (FM) and pumpkin leaf flour (PF) at 24 hrs and 48 hrs of fermentation time are shown in *figures 1–2*. Significant ($p < 0.05$) difference was observed in the mineral composition of 24 hrs and 48 hrs of fermentation time. The mean values of calcium ranged from 49.49 mg/100 g to 206.39 mg/100 g and from 51.86 mg/100 g to 202.90 mg/100 g for 24 hrs and 48 hrs of fermentation time respectively. Calcium increased significantly ($p < 0.05$) with increase in pumpkin leaf flour in the flour blends.

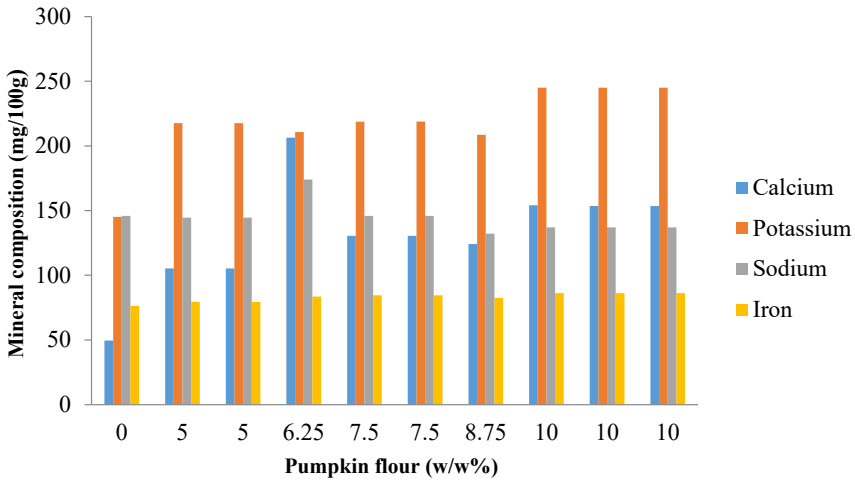

Figure 1. Mineral composition of 24 hrs fermented millet and pumpkin leaf flour

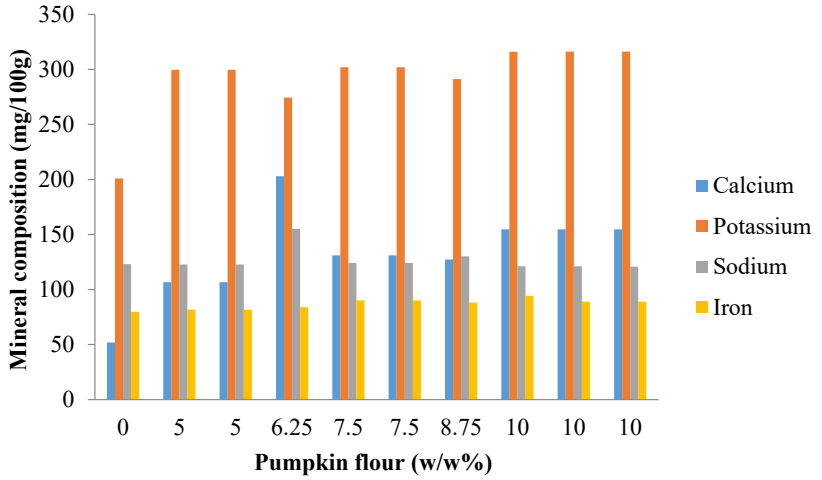

Figure 2. Mineral composition of 48 hrs fermented millet and pumpkin leaf flour

The regression coefficient of the flours at 24 hrs and 48 hrs of fermentation time are shown in *tables 1–2*. Their linear and interaction effect was not significant ($p > 0.05$) on the calcium content of fermented millet flour and fluted pumpkin leaves for 24 hrs and 48 hrs. The coefficients of determination (R^2) values were 0.51 and 0.70 for the flour blends of 24 hrs and 48 hrs of fermentation time respectively.

Potassium content for the fermented millet and pumpkin leaf flour at 24 hrs and 48 hrs ranged from 145.15 mg/100 g to 245.02 mg/100 g and from 200.92 mg/100 g to 316.11 mg/100 g respectively. Potassium increased significantly ($p < 0.05$) with increase in pumpkin leaf flour in the flour blends. The least value at both the 24 hrs and 48 hrs millet fermentation period was observed in 0% PF formulation blends, while the maximum values were observed at 10% PF formulation blends. In addition, the interaction effect of 24 hrs fermented millet flour and fluted pumpkin leaf had significant effect ($p < 0.05$) on the potassium content of the formulation blend. However, the linear effect of 48 hrs fermented millet flour and fluted pumpkin leaves, as well as the interaction effect had no significant effect ($p > 0.05$) on the potassium content of formulation blends. The coefficients of determination (R^2) values were 0.84 and 0.62 respectively for the flour blends of 24 hrs and 48 hrs fermented time. This indicates that the equation is a good fit in predicting the potassium content of the flour blend. There was at first a decrease and then later an increase in the potassium content of the formulation blends in both fermentation periods as the fluted pumpkin leaf flour decreased.

Table 1. Regression coefficient of mineral composition of fermented millet and pumpkin leaf flour at 24 hrs

Parameter	Calcium	Potassium	Sodium	Iron
A	120.03	218.41	149.49	79.81
B	147.57	243.17	134.56	85.80
AB	51.84	-71.33*	31.03	4.70
F-value	0.53	15.42	1.82	14.20
R^2	0.509	0.8371	0.5779	0.8256

Notes: * Significant at ($p < 0.05$); A: fermented millet flour, B: fluted pumpkin leaf flour, AB: interaction effects of fermented millet and pumpkin leaf flour, R^2: coefficient of determination.

Table 2. Regression coefficient of mineral composition of fermented millet and pumpkin leaf flour at 48 hrs

Parameter	Calcium	Potassium	Sodium	Iron
A	120.58	297.02	126.93	81.40
B	148.92	315.65	120.22	90.51
AB	48.96	-56.47	37.92	11.00
F-value	0.61	4.88	1.02	10.85
R^2	0.701	0.620	0.541	0.783

Notes: * Significant at ($p < 0.05$); A: fermented millet flour, B: pumpkin leaf flour, AB: interaction effects of fermented millet and pumpkin leaf flour, R^2: coefficient of determination.

The sodium content of fermented millet grains and pumpkin leaf flour ranged from 132.13 mg/100 g to 173.98 mg/100 g and from 120.70 mg/100 g to 155.10 mg/100 g for 24 hrs and 48 hrs of fermentation time respectively. Sodium decreased significantly ($p < 0.05$) with increase in pumpkin leaf flour in the blends. The least values were observed in 8.75% PF formulation blends, while the maximum values were observed in 6.25% PF formulation blends. The maximum values were observed in 93.75% FM and 6.25% PF formulation blends, whereas the least values were observed in 90% FM and 10% PF formulation blends. The main and the interaction effect showed no significant ($p > 0.05$) effect on the sodium content of formulation blend for 24 hrs and 48 hrs of fermentation time for millet flour and fluted pumpkin leaves. The coefficients of determination were 0.58 and 0.54 for the flour blends of 24 hrs and 48 hrs respectively.

The mean values of the iron content of the fermented millet grains and pumpkin leaf flour blends ranged from 76.30 mg/100 g to 86.19 mg/100 g and from 79.63 mg/100 g to 94.34 mg/100 g for 24 hrs and 48 hrs of fermentation

time respectively. Iron content increased significantly ($p < 0.05$) with increase in pumpkin leaf flour in the flour blends. The linear and interactive effect of 24 hrs and 48 hrs of fermentation time for millet flour and fluted pumpkin leaves does not show a significant level ($p > 0.05$) regarding the iron content of the formulation blends. The coefficients of determination (R^2) values were 0.83 and 0.78, respectively, for the flour blends of 24 hrs and 48 hrs of fermentation time for millet flour and fluted pumpkin leaves. This indicates that the equation is a good fit in predicting the iron content of the flour blend. There was a decrease in the iron content of the formulation blends in both fermentation periods as the fluted pumpkin leaf flour decreased.

The mean values for the anti-nutritional composition of the formulated blends of 24 hrs and 48 hrs fermentation time for millet grains and pumpkin leaf flour are shown in *tables 3–4*. Tannin content ranged from 0.089 to 0.162% and from 0.080 to 0.141% respectively.

Table 3. Anti-nutritional composition of fermented millet and pumpkin leaf flour at 24 hrs

PF (w/w%)	Tannin (%)	Trypsin inhibitor (%)	Total phenolic (%)	Phytate (%)
6.25	0.135 ± 0.00^{b}	0.024 ± 0.00^{a}	0.120 ± 0.00^{k}	0.004 ± 0.01^{bc}
8.75	0.134 ± 0.00^{h}	0.040 ± 0.00^{a}	0.106 ± 0.01^{g}	0.004 ± 0.01^{bc}
10.00	0.141 ± 0.11^{lm}	0.027 ± 0.00^{a}	0.111 ± 0.00^{i}	0.004 ± 0.01^{bc}
10.00	0.142 ± 0.00^{mn}	0.026 ± 0.00^{a}	0.112 ± 0.00^{ij}	0.004 ± 0.01^{bc}
5.00	0.138 ± 0.00^{i}	0.024 ± 0.00^{a}	0.107 ± 0.00^{gh}	0.004 ± 0.01^{bc}
7.50	0.161 ± 0.00^{o}	0.029 ± 0.01^{a}	0.119 ± 0.01^{k}	0.004 ± 0.00^{bc}
7.50	0.162 ± 0.00^{o}	0.029 ± 0.01^{a}	0.119 ± 0.01^{k}	0.006 ± 0.01^{bc}
10.00	0.143 ± 0.00^{n}	0.029 ± 0.00^{a}	0.113 ± 0.00^{j}	0.006 ± 0.01^{e}
5.00	0.139 ± 0.00^{ij}	0.025 ± 0.00^{a}	0.108 ± 0.00^{bc}	0.005 ± 0.00^{de}
0.00	0.089 ± 0.00^{a}	0.063 ± 0.00^{a}	0.075 ± 0.00^{a}	0.001 ± 0.00^{a}

Notes: Mean values with different superscripts within the same column are significantly different ($p < 0.05$); PF: pumpkin flour.

As shown in *Table 5*, the linear effect of 24 hrs fermentation time for millet and fluted pumpkin leaves, as well as the interaction had no significant ($p > 0.05$) effect on the tannin content of the blend formulation. However, the interaction of 48 hrs fermentation time for millet flour and fluted pumpkin leaves had a significant ($p < 0.05$) effect on the tannin content of formulation blend, as shown in *Table 6*.

Table 4. Anti-nutritional composition of fermented millet and pumpkin leaf flour at 48 hrs

PF (w/w%)	Tannin (%)	Trypsin inhibitor (%)	Total phenolic (%)	Phytate (%)
6.25	0.119 ± 0.00^{f}	0.021 ± 0.00^{a}	0.093 ± 0.00^{e}	0.003 ± 0.01^{a}
8.75	0.127 ± 0.00^{g}	0.032 ± 0.15^{a}	0.103 ± 0.00^{f}	0.003 ± 0.01^{a}
10.00	0.139 ± 0.01^{ij}	0.027 ± 0.00^{a}	0.076 ± 0.00^{bc}	0.003 ± 0.01^{a}
10.00	0.140 ± 0.01^{jk}	0.028 ± 0.00^{a}	0.077 ± 0.00^{cd}	0.004 ± 0.00^{bc}
5.00	0.109 ± 0.00^{c}	0.021 ± 0.01^{a}	0.106 ± 0.00^{g}	0.003 ± 0.01^{a}
7.50	0.111 ± 0.01^{d}	0.021 ± 0.01^{a}	0.119 ± 0.00^{k}	0.003 ± 0.01^{a}
7.50	0.113 ± 0.01^{e}	0.022 ± 0.01^{a}	0.120 ± 0.00^{k}	0.003 ± 0.01^{ab}
10.00	0.141 ± 0.00^{kl}	0.029 ± 0.00^{a}	0.076 ± 0.00^{d}	0.005 ± 0.00^{de}
5.00	0.110 ± 0.00^{c}	0.022 ± 0.01^{a}	0.107 ± 0.00^{gh}	0.004 ± 0.00^{bc}
0.00	0.080 ± 0.00^{a}	0.014 ± 0.00^{a}	0.060 ± 0.00^{a}	0.003 ± 0.01^{a}

Notes: Mean values with different superscripts within the same column are significantly different ($p < 0.05$); PF: pumpkin flour.

Table 5. Regression coefficient of anti-nutritional composition of fermented millet and pumpkin leaf flour at 24 hrs

Parameter	Tannin	Trypsin inhibitor	Total phenolic	Phytate
A	0.14	8.2985×10^{-3}	0.11	4.440×10^{-3}
B	0.14	0.049	0.11	4.612×10^{-3}
AB	0.049	0.20	0.030	2.019×10^{-4}
F-value	1.35	0.50	2.05	0.020
R^2	0.4102	0.7438	0.60	0.76

Notes: * Significant at $p < 0.05$); A: fermented millet flour, B: pumpkin leaf flour, AB: interaction effects of fermented millet and pumpkin leaf flour, R^2: coefficient of determination.

The coefficients of determination (R^2) values were 0.41 and 0.93, respectively, for the flour blends of 24 hrs and 48 hrs fermentation time for millet flour and fluted pumpkin leaves. This is an indication that the equation is a poor fit in predicting the tannin content of the flour blend of 24 hrs fermentation time for millet flour and fluted pumpkin leaves, but it can accurately predict the tannin content of the flour blends of 48 hrs fermentation time for millet flour and fluted pumpkin leaves. Tannin intake has been implicated to cause depletion in the digestive enzymes which are responsible for the secretion and production of endogenous protein,

the malfunctioning of the digestive tract, and the toxic effect resulting from the metabolites (*Jan et al.*, 2022).

Table 6. Regression coefficient of anti-nutritional composition of fermented millet and pumpkin leaf flour at 48 hrs

Parameter	Tannin	Trypsin inhibitor	Total phenolic	Phytate
A	0.11	0.014	0.10	3.514×10^{-3}
B	0.14	0.039	0.077	3.971×10^{-3}
AB	-0.039*	0.084	0.091*	-3.326×10^{-3}
F-value	40.34	0.48	10.87	1.92
R^2	0.9308	0.84	0.7838	0.5906

Notes: * Significant at $p < 0.05$); A: fermented millet flour, B: pumpkin leaves flour, AB: interaction effects of fermented millet and pumpkin leaves flour, R^2: coefficient of determination.

The value of the trypsin inhibitor for the formulated blends of 24 hrs fermentation time for millet and pumpkin leaf flour ranged from 0.024% to 0.063% resp. On the other hand, the mean values of trypsin inhibitor for the formulated blends of 48 hrs fermentation time for millet and pumpkin leaf flour ranged from 0.014% to 0.032%, resp., as shown in *Table 6*. The main effect of the 24 hrs and 48 hrs fermentation time for millet and fluted pumpkin leaf flour, as well as the interaction, had no significant ($p > 0.05$) effect on the trypsin inhibitor of flour blends. In addition, the R^2 values were 0.74 and 0.84 for both the flour blends at 24 hrs and 48 hrs respectively. This indicates that the equation is a good fit in predicting the trypsin inhibitor content of the flour blend. There was first an increase and then later a decrease in the trypsin inhibitor content of the formulation blends at 24 hrs and 48 hrs as the fermented millet flour increased. However, the fact that the trypsin inhibitors are heat-labile suggests that they can be destroyed through processing such as grinding and cooking (*Venter & van Eyssen*, 2001; *Adane et al.*, 2013).

The values of the total phenolic content for the formulated blends at 24 hrs fermentation time for millet grains and pumpkin leaf flour ranged from 0.075 to 0.120%. The least values were observed in 100% FM and 0% PF formulation blends, while the maximum values were observed in 93.75% FM and 6.25% PF formulation blends. The mean values of the total phenolic content for the formulated blends of 48 hrs fermentation time for millet grains and pumpkin leaf flour ranged from 0.060 to 0.120%. The maximum values were observed in 92.50% FM and 7.50% PF formulation blends, whereas the least values were observed in 100% FM and 0% PF formulation blends. The linear effect of 24 hrs fermentation time for millet flour and fluted pumpkin leaves, as well as the interaction, had

no significant ($p > 0.05$) effect on the total phenolic content of the formulation blend. However, the interaction of 48 hrs fermentation time for millet flour and fluted pumpkin leaves had a significant ($p < 0.05$) effect on the total phenolic content of flour blends. The coefficients of determination (R^2) values were 0.60 and 0.78 for the flour blends of 24 hrs and 48 hrs fermentation time for millet and fluted pumpkin leaf flour respectively. The total phenolic content was observed to increase gradually upon the substitution levels of fermented millet and thereafter decrease at higher substitution levels notwithstanding the varying fermentation periods. During fermentation, the factors' conditions (temperature, time, and pH), the microorganism species present as well as the grain type all have significant effect on phenolic compounds (*Jan et al.*, 2022).

The values for the phytate content of the flour blends of 24 hrs fermentation time for millet grains and pumpkin leaf flour ranged from 0.004 to 0.006%. However, the mean values for the phytate content of the formulated blends of 48 hrs fermentation time for millet grains and pumpkin leaf flour ranged from 0.003% to 0.005%. The linear effect of 24 hrs and 48 hrs fermentation time for millet flour and fluted pumpkin leaves, as well as the interaction, had no significant ($p > 0.05$) effect on the phytate content of formulation blend. Also, the coefficients of determination (R^2) values were 0.76 and 0.59, respectively, for the flour blends of 24 hrs and 48 hrs fermentation time for millet flour and fluted pumpkin leaves. There was a slight decrease in the phytate content of the formulation blends at 24 hrs as fermented millet flour increased and fluted pumpkin leaf flour decreased. However, the phytate content of the formulation blends at 48 hrs fermentation time for millet flour increased as the fluted pumpkin leaf flour decreased – it was observed to initially decrease and then later increase at higher levels of fluted pumpkin leaves.

Conclusions

The study shows the effect of fermentation time on minerals and anti-nutritional factors of flour blends from pearl millet and pumpkin leaves. The calcium, potassium, sodium, and iron content of the flour blends from fermented millet and pumpkin leaf flour increased significantly at 24 hrs and 48 hrs fermentation time, while tannin, total phenolic, and phytate decreased significantly at both 24 hrs and 48 hrs fermentation time. Calcium, potassium, and iron were maximized, while sodium tannin, trypsin inhibitor, total phenolic, and phytate were set to "none". However, the optimized solutions from the D-optimal design were fermented millet flour of 90.81% and pumpkin leaf flour of 9.19% at 24 hrs and fermented millet flour of 90.52% and pumpkin leaf flour of 9.48% at 48 hrs fermentation time respectively.

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Stress response in bacteria originated from dairy products

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Abstract. In some bacteria, the stress adaptation response, a defence mechanism against low pH, can also induce a number of physiological and genetic resistance mechanisms that provide advantages for bacteria to resist other environmental factors. This phenomenon is called cross-protection, which can potentially have serious consequences for food safety. In some fermented, acidified foods, low pH can provide a favourable environment for the growth of bacteria. Bacteria can adapt to acidic environments and become able to survive various factors that occur during storage and processing such as salt, antibiotics, or technological effects.

The microbiota of dairy products includes beneficial microorganisms, spoilage bacteria, and foodborne pathogens. The most common bacteria on various dairy products are *Escherichia coli*, *Enterococcus* sp., *Staphylococcus* sp., and *Bacillus* sp.

The aim of this research is to determine the resistance of the identified bacteria (16S rDNA-based bacterial identification) to antibiotics and osmotic pressure as a consequence of their possible defence mechanisms adapted to the acidic environment.

Keywords and phrases: cheese, acidic condition, osmoregulation, antibiotic resistance

1. Introduction

Stress refers to any adverse factor or condition that affects microbial growth and survival. Bacteria on food are exposed to various stress factors during food

production, processing, and storage, which can cause inactivation or adaptation of microbial cells. Stress factors include physical treatments such as heat, pressure, or osmotic shock, chemical treatments such as acids or disinfectants, and biological stresses such as bacteriological stresses, e.g. bacteriocins, bacterial contamination (*Begley & Hill*, 2015; *Ding et. al.*, 2022). Bacteria sense changes in their environment and respond with altered gene expressions. Stress adaptation changes the virulence properties of pathogens and contributes to *in vivo* survival during infection.

A number of studies have shown that inactivation of foodborne pathogens or various stressors can trigger adaptive mechanisms and even lead to cross-protection mechanisms. The widespread use of antibiotics, herbicides, and sanitizers can lead to antibiotic-resistant pathogens. These bacteria become tolerant to the stress factors of food processing with increased viability. Antibiotic-resistant zoonotic bacteria are transmitted from animals (their products) to humans through food or skin contact, which leads to the development of antibiotic resistance through gene transfer (*Woode et al.*, 2020). Acquired tolerance is the result of chromosomal mutations, phenotype development through horizontal gene transfer or co-resistance/cross-protection phenomena (*Oniciuc et. al.*, 2019). The exact molecular mechanisms of the stress adaptation in food-borne pathogens are not yet well known, but their understanding is essential for the development and implementation of effective control measures (*Begley & Hill*, 2015).

Sodium chloride is a fairly common food preservative for inactivating microorganisms. The presence of salt in food creates an osmotic gradient between the intracellular and extracellular environment of bacterial cells that may lead to cell death. However, many pathogenic bacteria evolve cellular response systems, which respond by altering cell morphology, modulating of regulatory genes, and expressing different proteins. Another survival strategy comprises the osmoprotectants that control the osmotic pressure (*Malakar et al.*, 2022).

The presence of certain antibiotic resistance genes in bacteria increases osmoregulation. Multi-drug-resistant *Listeria monocytogenes* were found to be more resistant to osmotic stress than strains resistant to a single antibiotic. Similarly, antibiotic-resistant *Staphylococcus aureus* shows resistance to osmotic stress (*Woode et al.*, 2020). The general stress responses include specific proteins that are activated as a reply for stresses protecting the cell against multiple stresses. The best-characterized general stress response is the alternative sigma factors.

In Gram-negative bacteria (*E. coli*, *Salmonella* sp., and *Vibrio* sp.), the alternative sigma factor RpoS regulates general stress responses, whereas in Gram-positive bacteria (*L. monocytogenes*, *Bacillus subtilis*, and *S. aureus*) the stress response is regulated by the alternative stress sigma factor SigB. These factors play a central role in the development of a coordinated response to stress and have been shown to be involved in tolerance to low pH, increased osmolarity, temperature, bacteriocins, antibiotics, ethanol, and prolonged starvation (*Begley & Hill*, 2015).

Prior to exposure to acidic conditions, some bacteria develop tolerance to acidified environments. Survival in acidic conditions is due to several molecular mechanisms including proton efflux pump, alteration of membrane composition, control of iron uptake, basification of the cytoplasm *(Dawan & Ahn*, 2022; *Xu et. al.*, 2022).

The aim of this research is to determine the resistance of identified bacteria (16S rDNA-based bacterial identification) to antibiotics and osmotic pressure as a consequence of their possible defence mechanisms adapted to acidic environments.

2. Materials and methods

Bacteria from different cheeses (caraway cheese, fresh cheese, feta-type cheese, cottage cheese, whey cheese, salted cheese, and cheddar) were isolated on different selective media such as Pseudomonas Agar Base for isolation of the genus *Pseudomonas*, Mannitol Salt Agar for the isolation of *Staphylococcus aureus*, TBX (Tryptone Bile X-glucuronide Agar) for the detection of *Escherichia coli* and coliforms.

A stock suspension was prepared in physiological solution (0.9% NaCl). 10 g of sample was weighed into 90 ml of physiological solution. 0.1 ml of each stock suspension was spread on the selective agar mediums and incubated at 37°C for 24 hours.

The molecular identification of the bacteria strains at the species level was performed by 16S rDNA sequence analysis (*Tóth et al.*, 2018; *György et al.*, 2022).

To determine the acid tolerance of the selected bacterial strains, bacterial cultures prior to incubation at 37°C for 24 hours were centrifuged at 6,000 g for 5 minutes. After centrifugation, the pellets were resuspended in nutrient broth with different pHs: 3, 3.5, and 5.5, adjusted with 1 M HCl and lactic acid, respectively, and incubated for 1 h at 37°C without shaking. After incubation, the cells were inoculated into nutrient broth at pH 7, and bacterial survival was determined by optical density (OD) at 595 nm after 24 hrs. The bacterial survival rate was calculated as follows: OD sample/OD control 100 incubation (*Horlbog et al.*, 2018; *Nath et al.*, 2020; *György et al.*, 2022).

To determine osmotolerance, bacterial strains were inoculated into nutrient broth containing 0%, 2%, 4%, 6.5%, 10%, 15%, and 20% NaCl. After incubation for 24 hrs at 37°C, the optical density (OD) of the bacterial culture was measured at 595 nm. Bacterial survival rate/growth rate was calculated as OD sample/OD control*100 (Horlbog et al., 2018; Nath et al., 2020; György et al., 2022).

The susceptibility of the selected identified bacteria to eight different antibiotics (ampicillin (AMP 25), kanamycin (K 30), clindamycin (CD 2), streptomycin (S 10), erythromycin (E 15), chloramphenicol (C 30), gentamicin (GEN 10),

tetracycline (TE 10)) was determined by the agar diffusion method. The size of the inhibition zones was measured. Based on the size of the inhibition diameters, the susceptibility or resistance of the bacteria was determined according to the recommendations of the European Committee for Antibiotic Susceptibility Testing (*EUCAST 2023*).

3. Results and discussions

The bacterial colony counts on selective media from commercial and artisanal dairy products obtained with different technologies are shown in *Table 1*. Bacteria were detected on Pseudomonas selective media only from caraway cheese. The bacterial counts of the dairy products tested ranged from $1.5 \cdot 10^2$ CFU/g for cottage cheese and from $2.68 \cdot 10^4$ CFU/g for feta-type cheese.

Table 1. Viable counts of some selected cheeses

Cheese samples	**Selective media**			
	Czapek – Dox	**TBX**	**Mannitol – Salt**	**Pseudomonas**
	CFU/g			
Caraway cheese	-	-	-	$6.5 \cdot 10^2$
Salted cheese	-	$8.8 \cdot 10^3$	$1.5 \cdot 10^2$	$1.45 \cdot 10^4$
Feta-type cheese	-	$4.9 \cdot 10^2$	$2.68 \cdot 10^4$	$7.6 \cdot 10^3$
Fresh cheese	$8.4 \cdot 10^2$	$1.12 \cdot 10^4$	$9.9 \cdot 10^3$	$1.76 \cdot 10^3$
Whey cheese	$< 10^3$	$7.3 \cdot 10^3$	$3.9 \cdot 10^3$	$2.8 \cdot 10^3$

Dairy products, including cheese and cottage cheese, are susceptible to microbial contamination because they provide ideal conditions for the growth and survival of microorganisms. Several studies summarize that improperly handled raw materials, non-hygienic and different production conditions contribute to microbial contamination, posing a health risk for consumers. *Studenica et al.* (2022) have shown in artisanal cheeses the presence of several pathogenic bacteria: *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella* sp., reaching a cell count 10^6. The isolated bacterial strains were identified based on 16S rDNA gene sequence similarity – sequences > 95% similarity were identified on the genus level, whereas sequences > 97% identity were identified on the species level (*Tóth et al.*, 2018; *Johnson et al.*, 2019).

The identified bacterial strains belong to different genera such as *Staphylococcus*, *Citrobacter*, *Bacillus*, *Actinobacteria*, *Alkalihalobacillus*, *Micrococcus*, and *Enterococcus* (*Table 2*).

Table 2. Identified bacterial strains ant their sequence similarity %

Bacterial strains	**Similarity (%)**
Staphylococcus saprophyticus	98.61
Citrobacter freundii	98.69
Enterobacter mori	87.14
Bacillus cereus	97.94
Bacillus sp.	99.72
Bacillus sp.	98.94
Bacillus sp.	98.95
Staphylococcus xylosus	99.07
Staphylococcus xylosus	99.42
Bacillus sp.	98.90
Actinobacterium sp.	97.04
Enterococcus faecalis	99.05
Bacillus toyonensis	98.35
Citrobacter youngae	98.91
Micrococcus endophyticus	96.40
Bacillus cereus	99.63
Alkalihalobacillus clausii	95.52
Bacillus licheniformis	97.31
Bacillus sp.	98.53
Bacillus thuringiensis	99.34

The two main sources of bacteria in cheese are the starter culture and the raw milk microbiota. However, cheese can be contaminated with microorganisms from the processing environment, sometimes affecting the ripening and the organoleptic properties of the product. Bacterial strains are essential for the development of cheese characteristics and are responsible for organoleptic properties such as flavour (*György & Laslo*, 2021). The composition and production technology of cheese is extremely diverse. The quality and processing of the milk, the environmental conditions (temperature, humidity, salinity), and the technological parameters used influence the prevalence, quantity, and diversity of microbial species. There are more microbial species on the surface of the cheese than in the inside of

the product. The most commonly found bacteria on cheese are *Escherichia coli*, *Enterococcus* sp., *Shigella* sp., *Proteus* sp., *Staphylococcus* sp., *Bacillus* sp., and *Listeria* sp. It has been shown that the surface of cheese is characterized by aerobic bacteria such as *Brevibacterium* sp., *Bacillus sp.*, and *Micrococcus sp*. The predominant microbes inside the cheese are anaerobic or facultatively aerobic microorganisms such as *Escherichia coli* (*György & Laslo*, 2021).

Amplicon-based sequencing of 16S rRNA revealed that *Streptococcus* sp. species were most prevalent in Provolone (72–85%) and Swiss cheese (60–67%), whereas *Lactococcus* sp. species were dominant in Cheddar cheese (27–76%). Species diversity varied considerably. Alpha diversity analysis showed that soaked Cheddar cheese had the highest heterogeneous microbial diversity, while smoked Provolone cheese had the lowest. The microbial diversity of the cheese rind region was higher than that of the core region, as the smoking and soaking processes affected the rind of each cheese. Within a given cheese type, the microbial composition was very similar regardless of the sampling location (*Choi et al.*, 2020). Changes in the acidity of cheese stimulate the growth of certain coliforms and foodborne pathogens. The presence of coliforms in white ripened cheese is responsible for premature bloating and the formation of large gas holes in the cheese mass (*Hayaloglu, 2016*; *György & Laslo*, 2021).

The smear-ripened cheese microflora contained mainly coryneform bacteria, followed by staphylococci and micrococci, which were able to grow in the presence of 10% NaCl. The most common staphylococci were the *Staphylococcus equorum*, *S. saprophyticus*, *S. caseolyticus*, and *S. xylosus* (*Hayaloglu*, 2016). Our results regarding the identified bacteria were in agreement with *Martin et al's* 2021 results – similar bacteria were found in cheeses such as *Enterobacter aerogenes*, *C. braakii*, and *C. freundii*. Coliforms are indicator bacteria resulting from poor hygienic conditions. They are responsible for premature bloating of cheeses causing gas formation and deformation of the cheese paste. The presence of spore-forming bacteria are associated with the raw milk environment. The presence of the potentially pathogenic bacteria *Citrobacter freundii* has been detected in several cheeses and dairy products (*Gaglio et al.*, 2021; *Gücükoğlu et al.*, 2023).

The inorganic (hydrochloric acid, HCl) and organic (lactic acid) acid stress tolerance of identified bacteria at 37°C was determined by preliminary adaptation step. The frequent use of low pH as an additive or preservative in foods may exert a selective pressure on bacterial growth, leading to acid tolerance in bacterial strains. The acid tolerance of bacterial strains was characterized by determining the survival rate (*Table 3*). The analysed pH values resulted in different survival rates. At pH 5.5, adjusted with lactic acid and HCl, bacterial growth was almost above 50%. Some of the tested bacteria survived the acid shock, and some of them are adaptive acid-tolerant such as *Staphylococcus xylosus* or *Bacillus* sp.

At pH 3, adjusted with HCl, *Staphylococcus saprophyticus* showed a survival rate of 50.15% and *Enterococcus faecalis* 82.47%. Some of the *Bacillus* species showed good survival rate at the tested pHs.

Table 3. Identified bacterial strains survival rate (%) in acidic conditions

Isolated bacterial strains	pH – 3 HCl	pH – 5,5 HCl	pH – 3,5 Lactic acid	pH – 5,5 Lactic acid
	Survival rate (%)			
Staphylococcus saprophyticus	50.15	55.41	6.46	71.02
Citrobacter freundii	37.78	60.56	8.89	79.82
Enterobacter mori	48.88	56.86	12.72	59.23
Bacillus cereus	15.71	53.68	24.55	60.64
Bacillus sp.	45.08	70.86	11.03	67.75
Bacillus sp.	134.44	98.89	25.56	437.78
Bacillus sp.	20.54	62.68	36.74	62.79
Staphylococcus xylosus	55.90	88.33	7.95	80
Staphylococcus xylosus	54.10	75.84	18.54	89.818
Bacillus sp.	51.17	100	17.58	100
Actinobacterium sp.	9.81	45.61	12.36	56.18
Enterococcus faecalis	82.47	50.52	5.73	56.08
Bacillus toyonensis	47.63	54.50	6.41	78.78
Citrobacter youngae	31.64	66.98	10.90	52.80
Micrococcus endophyticus	39.73	61.33	34.52	61.04
Bacillus cereus	44.09	45.90	9.32	51.04
Alkalihalobacillus clausii	35.94	15.40	9.60	80.58
Bacillus licheniformis	61.87	82.58	12.07	66.26
Bacillus sp.	60.20	48.33	17.80	57.73
Bacillus thuringiensis	39.23	51.21	11.74	59.81

Horlbog et al. (2018) reported that resistance to acid is an important factor in the survival and infection of pathogenic bacteria. Strains belonging to *Enterococcus* genus are acid-tolerant and maintain the intracellular pH homeostasis (*Gaca & Lemos*, 2019). In *Enterobacteriaceae*, acid shock induces acid shock proteins. Gram-positive bacteria respond to the acid and osmotic stress with responsive σb and σ3 factors (*Marmion et al.*, 2022). Acid tolerance response is determined by different environmental and growth factors, also the type of the acid (*Xu et al.*, 2022).

The NaCl tolerance (0%, 2%, 4%, 6.5%, 10%, 15%, and 20%) of the tested bacterial strains was characterized by survival rates (*Table 4*). In the presence of

2–6.5% NaCl, the growth of *Staphylococcus xylosus* was moderate. The higher concentrations of NaCl inhibited growth completely.

10% and 15% of NaCl inhibited the growth of the isolated bacterial strains, except for *Bacillus sp.*, *Staphylococcus xylosus*, and *Staphylococcus saprophyticus*, where the growth was lower.

Li et al. (2021) reported that increased NaCl concentrations inhibited the growth patterns of bacterial species. The salt tolerance, osmoregulation of bacteria is a complex process involving increased uptake of potassium ions or the production of various metabolites. The use of NaCl for spoilage prevention is based on the low resistance of spoilage and pathogenic bacteria to high osmotic pressure. Adaptation to higher salt concentrations has recently been described for a number of bacteria, including those that are harmful to human health such as *Bacillus cereus* or *Enterococcus faecalis*.

Table 4. Survival rates (%) in the presence of NaCl of the selected bacterial strains

Bacterial strains	**Survival rate (%)**					
	2%	**4%**	**6.5%**	**10%**	**15%**	**20%**
Staphylococcus xylosus	31.94	30.69	58.04	1.04	1.88	2.71
Actinobacterium sp.	67.41	74.66	40.86	32.07	18.97	0.86
Citrobacter freundii	58.24	114.78	18.63	11.78	3.64	1.28
Bacillus cereus	53.01	56.93	67.17	61.14	59.64	25.30
Citrobacter youngae	65.09	43.39	18.20	11.47	13.22	5.49
Staphylococcus xylosus	100.46	89.66	90.11	55.63	39.08	4.60
Enterococcus faecalis	97.16	94.54	65.28	19.43	6.55	0.87
Staphylococcus saprophyticus	47.44	53.91	69.00	49.60	39.35	1.08
Alkalihalobacillus clausii	31.43	28.57	54.90	7.35	2.45	0.00

Bacterial strains with antibiotic resistance could be a source of antibiotic resistance genes. Bacterial susceptibility/resistance was determined according to the EUCAST recommendations based on the diameter of the inhibition zones.

Resistance to ampicillin was found in *Citrobacter freundii* isolated from fresh cheese and in *Staphylococcus saprophyticus* and *Alkalihalobacillus clausii* as originated from cottage cheese.

Resistance to tetracycline was detected in *Bacillus* sp. isolated from cottage cheese and whey cheese and in *Enterococcus faecalis* and *Alkalihalobacillus*

clausii as originated from cottage cheese. Metagenomic analysis of tetracycline resistance in cheese bacteria summarized that raw-milk cheeses were considered a source of tetracycline resistance genes that could be shared via horizontal gene transfer (*Flórez et al.*, 2017).

Resistance to clindamycin was detected in *Citrobacter youngae* isolated from whey cheese, in two *Staphylococcus xylosus* isolated from Cheddar and fresh cheese, and in *Bacillus* sp. isolated from salted cheese.

Resistance to erythromycin was found in two *Bacillus sp.*, in *Enterococcus faecalis* from cottage cheese, and in *Staphylococcus xylosus* from fresh cheese. Enterococci with antibiotic resistance genes, virulence genes, and biofilm-forming capacity were found in traditional dairy products as a result of poor food hygiene practices (*Amidi-Fazli & Hanifian*, 2022).

Citrobacter freundii from whey cheese and *Alkalihalobacillus clausii* from cottage cheese showed resistance to streptomycin.

Alkalihalobacillus clausii and a *Bacillus* sp. were also resistant to kanamycin, and *Alkalihalobacillus clausii* was resistant to chloramphenicol and gentamicin.

The broad spectrum of resistance was observed in the case of *Alkalihalobacillus clausii*, which was susceptible to only two tested antibiotics. *Alkalihalobacillus clausii,* with the old name *B. clausii*, was detected in ropy bread spoilage. Strains of these bacteria with probiotic potential possessed genes encoding multiple antibiotic resistance, but these genes were not located on mobile elements (*Pacher et al.*, 2022; *Dhakephalkar et al.*, 2022).

The overuse of antibiotics for prophylaxis or treatment of infectious diseases is connected with the emergence of antibiotic resistance and the dissemination of this phenomenon. Resistant bacteria can be transmitted through the consumption of livestock products, milk, or related foods. Types of antibiotic resistance are intrinsic and acquired. Acquired antibiotic resistance can result from mutation and horizontal gene transfer (conjugation, transformation, transduction). Dairy products, including fresh cheese, have been shown to contain phage particles with antibiotic resistance genes (*Blanco-Picazo et al.*, 2022).

4. Conclusions

The microbial analysis of tested cheeses showed high microbial load. However, the results are in line with microbiological limits; among and identified bacteria, there are pathogenic and spoilage bacteria. These bacteria are *Bacillus cereus*, *Citrobacter youngae*, *Staphylococcus xylosus*, *Enterococcus faecalis*, and *Staphylococcus saprophyticus*. Cheeses have been contaminated with the undesirable bacteria that are resistant to certain antibiotics, either through cross-contamination or as a result of poor hygienic conditions. Resistance to stress factors that may

appear during food production, such as different NaCl concentrations and pH, are characteristics of bacteria strains with antibiotic resistance. This is probably explained by the phenomenon of cross-protection. A further aim is to demonstrate the resistance of the bacterial strains to other food-related stresses.

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Development and comparative analysis of protein-polyphenol-fibre bars as nutritional supplements suitable for healthy senior consumers

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Abstract. The number of elderly people is steadily increasing in developing countries though the specific age-related challenges of nutrition fail to get properly addressed in the case of senior citizens. Accordingly, we have developed protein-polyphenol dietary fibre (PPF) bars using two kinds of protein mixtures (1 and 2) and some food additives, such as the banana powder, freeze-dried strawberries, coconut powder, Dutch cacao powder, and vanilla cookies, as they can interfere with the texture of bars and the flavour as well. The used food additives are also a source of polyphenols and dietary fibres that would enhance the nutritive values of the bars. The texture properties, such as hardness and cutting force, were assessed, and the results indicated a significant difference ($P < 0.05$) among the bars, offering important hints about their suitability for the elderly. Also, significant differences were observed for the polyphenol content of the bars that would stress their increased nutritional relevance too. On average, the sensorial evaluation showed the developed bars of moderate acceptability, while Bar 3 and 6 had

the highest scores for colour, texture, flavour, and aroma. Conversely, Bar 1 recorded the lowest values for all assessed criteria.
Interestingly, Bar 3 with freeze-dried strawberries and Bar 6 with Dutch cacao powder were the most appreciated flavours and contained in the range of 25–28% protein, 17–23% carbohydrate, 15–21% lipids, and 15–23% dietary fibre, which also indicates their nutritionally balanced nature. Furthermore, the above-mentioned macronutrient content ensures approximately 400 Kcal/100g per PPF bar, while through their polyphenol and flavonoid yield their health-promoting effect gets substantiated.

Keywords and phrases: protein bars, senior consumer, nutritional supplement, antioxidant activity, texture profile analysis

1. Introduction

Population ageing is becoming an important issue with relevant societal and economic implications. The world population in mid-2022 reached 7,963 million, while the people over the age of 65 were representing 10% of them (*Population Reference Bureau*, Datasheet 2022). At the same time, Europe's population had grown to 742 million, and 19% of the population was over the age of 65.

Moreover, as we grow older, our health condition seems to deteriorate due to inflammageing, a phenomenon featuring increased levels of serum-specific inflammatory markers, which are associated with a high susceptibility to different chronic diseases and premature death (*Ferrucci & Fabbri*, 2018).

In normal circumstances, as adults advance in age, their nutrient needs change, so elderly people need more and/or different nutrients than younger adults, speaking in general terms (*Panda & Booth*, 2022). Additionally, chronic illnesses, medications, and a decrease in physical activity can all increase the risk of nutrient deficiencies or unique requirements *(Baugreet et al.*, 2017). The predicted rise in the elderly cohort represents an opportunity and an obligation to develop optimized, high-protein-containing foodstuffs that could overcome the incidence of age-related conditions such as sarcopenia, one of the most common age-related problems (*Dao*, 2020). Consumption of at least 1.0 to 1.2 g protein/kg/bodyweight/day is recommended for healthy older adults aged 65+ in Europe (*Bauer et al.*, 2013). Moreover, besides proteins, it is equally important to pay attention to the polyphenol intake, as their health-promoting and anti-ageing effects have been demonstrated (*Yessenkyzy et al.*, 2020). Polyphenols can neutralize free radical species and chelate redox-active transition metal ions, all having an implicit antioxidant effect. Furthermore, they can modulate the expression of pro- and anti-apoptotic genes and activate genes implicated in the antioxidant stress response via the Keap1-Nrf2-ARE signalling pathway (*Liu et al.*, 2022). Polyphenols also affect mitochondrial functions, prevent protein aggregation, and induce autophagy

having a caloric restriction mimetic effect, and they can be used to prevent or counteract many age-related diseases such as inflammation, diabetes, cancer, and neurodegeneration (*Madeo et al.*, 2019). Polyphenols can also interact with the microbiota–gut–brain axis affecting the ageing brain functionality (*Sarubbo et al.*, 2023).

In recent times, nutritional protein bars have become increasingly popular worldwide due to their convenience and ease of consumption (*Goodbody*, 2013). Typically, they are made from a blend of ingredients like oats, nuts, dried fruits, and protein, providing a nutrient-dense snack or meal option. Protein bars are generally low in calories and fat, making them a healthier choice for snacking in case of children and middle-aged people. Moreover, they are available in various flavours, offering the above-mentioned consumers a wide range of options to choose from (*Padmashree et al.*, 2012). Lately, there has been a rising trend in the utilization of plant proteins as a cost-effective and adaptable substitute for animal-derived sources in human diets. Furthermore, the incorporation of plant proteins into various food uses, including the production of high-protein bars, has the potential to enhance the appeal of these items to individuals following vegan, vegetarian, or active lifestyles (*Sá et al.*, 2020). Overall, rice protein is a good option for people who are looking for a plant-based protein source that is low in carbohydrates and fat. However, it may not provide the same range of nutrients as some other plant-type protein sources, so it is important to consume a diversified diet that includes a variety of protein sources. Moreover, the hydrolysis of proteins with proteases would produce many potential peptide sequences and amino acids providing plenty of nutritive properties. It could also enhance the antioxidative properties of native proteins by attacking the peptide bonds in the interior of polypeptide chains producing a range of polypeptides that differ in molecular weight or amino acid sequences (*Phongthai et al.*, 2017). Further to rice, pea protein has attracted significant attention due to its minimal allergenic potential, exceptional nutritional value, ready availability, and affordability similar to other plant proteins. It contains high levels of lysine but tends to be limited to methionine and tryptophan. Hence, pea proteins are frequently paired with cereal grains because they offer a complementary essential amino acid profile, addressing the typical deficiency in lysine found in cereal proteins, but they contain higher levels of sulphur amino acids (methionine, cysteine) (*Lam et al.*, 2018). Besides rice and pea, the sunflower protein exhibits promising potential as a functional protein owing to its favourable solubility characteristics. Typically derived as a by-product of oil extraction, it often undergoes denaturation during processing, resulting in diminished solubility and functionality. However, when protein fractions are isolated without denaturation, sunflower proteins have the capacity to display

solubility across a range of ionic strength and pH levels, making them suitable to provide amino acids (*Ermiş & Karasu*, 2020). Another source for proteins is the hemp whose protein pool features several health-promoting properties, e.g. they influence the angiotensin I-converting enzyme and acetylcholinesterase inhibitions (*Wang & Xiong*, 2019). The hemp protein extract shows antioxidant, hypocholesterolaemia, and hypoglycaemic effects, having significant amounts of arginine and glutamine.

Besides proteins and polyphenols, another important dietary component that has significant relevance for antiaging are the dietary fibres (*Yu et al.*, 2022). The observed and/or recommended intake of dietary fibres varies among people with different cultural backgrounds, so the Iranian elderly would have 25 g/day (*Safarnavadeh et al.*, 2023), Slovenian elderly people consume on average 22.4 g/day (*Seljak et al.*, 2021), while in a USA study people were ingesting 11–21 g/day (*Shivakoti et al.*, 2022). The fibre intake has been correlated with the gastrointestinal and mental health by defining the microbiota–gut–brain axis (*Berding et al.*, 2021), while the mentioned axis implications in neurodegenerative and neuropsychiatric disorders are currently studied intensively (*Bicknell et al.*, 2023; *Shobeiri et al.*, 2022).

It seems, therefore, likely that senior citizens have specific requirements when it comes to healthy nutrition that aids prevention of inflammageing and chronic diseases. It is also important to bear in mind that the maintenance of physical, physiological, and cognitive activities at any time in our lives is an extraordinarily complex task that would require not only the implication of conscious consumers themselves but healthcare professionals too. However, the collaboration of nutritionists, food scientists, and food manufacturers would be more than welcome since they are also responsible for providing suitable foodstuff for people including the elderly (*Alden-Nieminen et al.*, 2009; *Tedre & Pehkonen*, 2014).

The objective of the currently reported study was to develop some protein-, polyphenol-, and dietary-fibre-containing foodstuff that we had named PPF bars and tested them for selected structural, physicochemical, and nutritional parameters. We also evaluated the effects of different protein sources (sunflower, rice, hemp, and pea) in the context of the PPF bar's acceptability by a larger cohort of consumers. We must admit that the presented results represent the initial phase of a larger project, which is meant to result in foodstuff that would support more efficiently the special nutritional requirements of healthy senior people.

2. Materials and methods

All raw materials, such as protein powders, vegetable oil (rapeseed oil, sunflower oil), coconut powder, Dutch cocoa powder, cookies, vanilla powder, maltodextrin, soy lecithin, freeze-dried strawberries and bananas, were purchased from the local markets.

The soy protein isolate, pea protein isolate, rice protein concentrate, sunflower protein, and hemp protein were purchased from an organic shop and were produced by Nutriversum Ltd., Hungary; the glucose syrup (Dextrose Equivalent "DE 38") was a product of KALL Ltd., Hungary, the vegetable oils (rapeseed oil, sunflower oil) were from Bellasan Ltd. (ALDI, Hungary), maltodextrin from VitalTrend Ltd., Hungary, soy lecithin was supplied by Brenntag Ltd., Hungary, natural vanilla aroma in powder by Dr. Oetker Ltd., Hungary cookies by Győri Ltd., Mondelez Hungary, Dutch cacao powder by Dr. Oetker, Hungary, freeze-dried strawberries by Viblance Ltd., Hungary, and coconut powder by Happy Harvest Ltd. (ALDI, Hungary).

The technical and food safety aspects of the products, such as the batch numbers, physicochemical composition, presence of recognized food allergens, sensory characteristics (such as appearance, flavour, and aroma), microbial content, and shelf life, were all checked as well.

Banana powder preparation

At the local market, fully ripe bananas were obtained and sliced into 0.5-cm-thick pieces with the peel intact. To minimize enzymatic browning, the banana slices were immersed in a 10% citric acid solution for 10 minutes. Subsequently, the peel was removed, and the banana slices were air-dried to reduce excess moisture. The dried banana slices were then placed in a cabinet dryer at 60°C for 5 hours, after which they were ground using a Panasonic Mixer Grinder MX-AC555 (India) and sieved through a 30-mesh screen (0.595 mm) (Cole-Parmer, Germany) to obtain a fine flour. Finally, the banana flour was vacuum-sealed for future applications and stored at a 4°C fridge until being used (*Ovando-Martinez M et al.*, 2009).

Preparation of Protein Bars

The optimal amounts of banana powder, coconut flour, Dutch cocoa powder, Oreo cookies, freeze-dried strawberries, salt, and lecithin were determined through trial-and-error methods to achieve the desired colour and texture

of the bars. Additionally, the remaining ingredients were selected based on consumer preferences as determined by a survey (data not presented). Once all the ingredients for the recipe were combined, the resulting mixture was placed into silicone moulds and cooled under refrigeration conditions at 4°C for 4 hours. The bars were then coated with chocolate and cooled. Each bar, weighing 40 g, was cut into a rectangular shape and placed in metalized polyester polyethylene (MET-PPE) packaging, which was then sealed. The formulations of the bars were adjusted so that consuming one bar per day could provide the elderly with approximately 23% and 25% of their recommended daily allowance (RDA) of proteins and calories respectively. The rough composition (protein mixture and flavour-conferring ingredient) of PPF bars is presented in *Table 1*. There were two protein mixtures used for the formulation of recipes: Mixture 1, which included pea protein isolate and rice protein, and Mixture 2 containing pea protein isolate, rice protein, hemp protein, and sunflower protein. In *Table 1*, there are also indicated the flavouring ingredients that could also contain important macro- and micronutrients together with phytonutrients and dietary fibres.

Table 1. Rough composition of PPF formula

Bar symbol	Protein mixture and flavour
Bar 1	Mix 1 + Banana powder
Bar 2	Mix 1 + Dutch cacao powder
Bar 3	Mix 1 + Freeze-dried strawberries
Bar 4	Mix 1+ Vanilla and cookies
Bar 5	Mix 2 + Coconut powder
Bar 6	Mix 2 + Dutch cacao powder
Bar 7	Mix 2 + Freeze-dried strawberries
Bar 8	Mix 2 + Vanilla and cookies

Proximate composition analysis and caloric value determination

The composition of PPF bars including moisture, protein, and lipid contents were determined according to the prescribed methods of AOAC (*Association of Analytical Chemists*, 2019), and the results were expressed on a dry-weight basis. The total caloric values (kcal) of the ingredients and samples of final products were calculated as mentioned by *AOAC* (2019), according to the following equation:

$$\text{Energy (kcal)} = [\text{Protein (g)} \times 4] + [\text{Carbohydrates (g)} \times 4] + [\text{Fat (g)} \times 9]. \quad (1)$$

The determination of the total phenolic and flavonoid content

Determination of the total polyphenol content

Total phenolic content (TPC) was measured by Folin–Ciocâlteu's spectrophotometric method at 760 nm, according to *Lamuela-Raventós* (2018). The content of all phenolic compounds in the tested samples were determined using a calibration curve, for which gallic acid is used (mg GAE /100g). All analyses were performed in triplicate, and the results were statistically evaluated by Excel, a Microsoft Office software program.

Determination of flavonoid content

Total flavonoid content (TFC) was measured spectrophotometrically at 510 nm according to *Seifu et al.* (2017). The content of all flavonoid compounds in the tested samples were determined using the calibration curve, for which catechin was used. The result is given in terms of mg catechin/100 g product. All analyses were performed in triplicate and the results were statistically evaluated by Excel, a Microsoft Office software program.

Texture analysis

A texture analyser, TA (TA – XT2 plus, Stable Micro Systems, Surrey, U.K) was used to measure the hardness of the PPF bar samples as described earlier (*Yuan & Chang*, 2007).

Penetration tests

A single-penetration force-versus-time program was used to compress along the sample thickness at a test speed of 0.50 mm/s and return to its original position. The original clearance between the probe and the base in the machine's load cell was 0.5 mm, so when the probe moved down, it would compress the test sample held horizontally against the base to 0.500 mm, and the hold time was 30 sec. The program software was set to move the probe at 1.0 mm/sec for the pre-test and at 10.00 mm/sec for the post-test phase. A stainless steel probe with a diameter of 10 mm (P/5) was used to compress one sample. The peak force indicated by the force time curve was taken as the maximum compressive force/hardness.

Cutting/shearing tests

The cutting force of the of PPF bars was measured using Texture Analyser (TA-XT2 plus); a single-cutting force-versus-time program was used to compress along the sample thickness at a test speed of 2.00 mm/s and return to its original position. The original clearance between the probe and the base in the machine's load cell was 0.5 mm, so when the probe moved down, it would cut the test sample held horizontally against the base to a distance of 20 mm. The program software was set to move the probe at 1.0 mm/sec for the pre-test and at 10.00 mm/sec for the post-test phase. A stainless steel blade set with knife (HDP/BSK) comprising a Warner Bratzler blade (a reversible blade with knife edge) with a slotted blade insert and a blade holder was used to cut one sample. The cutting curve was obtained by recording the maximum force the blade needs to cut the sample completely. The results were based on the maximum peak (maximum force) resulting from the shear stress.

Sensory evaluation

Sensory evaluation was performed by trained panellists who were experienced in sensory evaluation using the 9-point hedonic scale. The panellists were asked to assess the product based on various criteria, such as appearances, flavour, aroma, colour, texture, and overall acceptability, by choosing and marking one of nine alternatives (ranging from 1 = like extremely to 9 = dislike extremely) according to *Banach et al.* (2014). Instructions were provided before they evaluated the PPF bars. During tasting sessions, samples were presented randomly to panellists. Eight coded samples were presented to the participants. They were instructed to rinse their palate with water between samples. Samples were portioned into equal weights, placed in plastic plates, and kept at room temperature (22 ± 1 °C) for 30 min before analysis. The evaluations were carried out in two sessions.

Statistical analysis

The statistical analysis was performed with the help of one-way analysis of variance (ANOVA) using Minitab statistical analysis software. Differences among obtained means were tested by Tukey's honestly significant difference test (Tukey's HSD). Results are expressed as means ± standard deviation of replicated samples.

3. Results and discussion

The development of PPF bars was carried out as described in the *Materials and methods* section. There have been obtained eight types of bars of which samples 1–4 contained Protein Mix 1 (pea and rice protein isolates in equal amounts) and samples 5–8 having Protein Mix 2 (equal amounts of pea protein isolate, rice protein, hemp protein, and sunflower protein extracts). The detailed composition of the eight PPF bars is shown in *Table 2*. In this table, all the ingredients are indicated, and many of them are expected to confer specific features to every sample. Among these ingredients, the so-called flavours, i.e. the banana powder, coconut powder, Dutch cocoa powder, cookies, freeze-dried strawberries, soy lecithin, and vanilla, were the most relevant. The maltodextrin was not of the resistant type, and its inclusion in the formulation of PPF bars had the purpose of facilitating the formation of an internal texture structure and sensorial quality that appeared more palatable for any age-specific consumer, including the elderly. It was our best intention to pay attention to the age-specific aspects of saliva that are crucial to the oral processing of food and are subsequently also related to sensory and textural experience (*Xu et al.*, 2019).

Table 2. The formulation of the PPF bars

Ingredients (g/100 g)	**Bar 1**	**Bar 2**	**Bar 3**	**Bar 4**	**Bar 5**	**Bar 6**	**Bar 7**	**Bar 8**
Protein Mix 1	33.00	33.00	33.00	33.00	0.0	0.0	0.0	0.0
Protein Mix 2	0.0	0.0	0.0	0.0	33.00	33.00	33.00	33.00
Banana powder	6.23	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Coconut powder	0.0	0.0	0.0	0.0	6.23	0.0	0.0	0.0
Dutch Cocoa powder	0.0	6.23	0.0	0.0	0.0	6.23	0.0	0.0
Cookies	0.0	0.0	0.0	10.23	0.0	0.0	0.0	10.23
Freeze-dried strawberries	0.0	0.0	10.23	0.0	0.0	0.0	10.23	0.0
Sunflower oil	2.4	2.4	2.4	2.4	2.4	2.4	2.4	2.4
Glucose syrup	31.82	31.82	27.82	27.82	31.82	31.82	27.82	27.82
Rapeseed oil	13.64	13.64	13.64	13.64	13.64	13.64	13.64	13.64
Maltodextrin	5.45	5.45	5.45	5.45	5.45	5.45	5.45	5.45
Water	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Soy lecithin	0.85	0.85	0.85	0.85	0.85	0.85	0.85	0.85

Ingredients (g/100 g)	Bar 1	Bar 2	Bar 3	Bar 4	Bar 5	Bar 6	Bar 7	Bar 8
Vanilla	0.91	0.91	0.91	0.91	0.91	0.91	0.91	0.91
Salt	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2

However, in the future, the use of resistant maltodextrin should also be considered, which is a soluble dietary fibre ingredient whose physiological functions are well studied, and its implications in regulating blood glucose levels and serum lipids are demonstrated (*Astina & Sapwarobol*, 2019).

The nutritional facts: Per/100 g bar

Next, we have analysed the proximate composition of the developed PPF bars, which are presented in *Table 3*. The assessment of the nutritional composition comprised parameters such as fat, carbohydrates, fibres, protein, and total caloric content, and the obtained data refers to 100 g of a given PPF bar.

Table 3. The proximate composition data of developed PPF bars

Bar symbol	Fat (g/100g)	Carbohydrate (g/100g)	Fibre (g/100g)	Protein (g/100g)	Energy, Kcal
	Mean + St. Dev.	Mean + St. Dev.	Mean + St. Dev.	Mean + St. Dev.	Mean + St. Dev.
Bar 1	15.27 ± 0.83^{b}	23.52 ± 0.55 ^{a}	15.97 ± 1.01^{b}	28.02 ± 0.03 ^{a}	341.38 ± 4.12^{a}
Bar 2	20.73 ± 2.04 ^{a}	17.55 ± 0.623 $^{b\,c}$	23.67 ± 0.91^{a}	26.83 ± 0.96^{a}	354.37 ± 2.01^{a}
Bar 3	20.50 ± 0.79 ^{ab}	19.27 ± 0.95 ^{b}	23.53 ± 0.56^{a}	25.1 ± 2.36^{a}	321.08 ± 1.00^{a}
Bar 4	19.37 ± 0.77 ^{ab}	19.33 ± 0.62 ^{b}	23.33 ± 1.78 ^{a}	26.75 ± 0.86^{a}	341.35 ± 3.15^{a}
Bar 5	20.54 ± 1.81$^{a\,b}$	15.54 ± 0.78 c	21.84 ± 2.62 ^{a}	27.06 ± 0.97^{a}	387.86 ± 3.57^{a}
Bar 6	21.0 ± 1.83 ^{a}	22.69 ± 0.34^{a}	21.98 ± 1.66^{a}	28.63 ± 0.77^{a}	368.06 ± 4.00 ^{a}
Bar 7	20.40 ± 1.71 ^{ab}	18.38 ± 1.08^{b}	23.12 ± 0.73 ^{a}	27.32 ± 0.29^{a}	377.80 ± 0.17^{a}
Bar 8	21.43 ± 3.71 ^{a}	18.36 ± 0.53 ^{b}	23.01 ± 0.40^{a}	27.46 ± 0.73^{a}	376.85 ± 2.41^{a}

Notes: * Values are expressed as mean ± SD. For each column, values that contain different letters in superscripts were significantly different ($P \leq 0.05$).

The fat content ranged from 15.2 g to 21.4 g. The results showed that the highest ($p < 0.05$) fat content (21.43 ± 3.71 g) was recorded for the sample *Bar 8* followed by the samples *Bar 6* and *Bar 2* (21.0 ± 1.83 g, 20.73 ± 2.04 g), and this fact could be due to the Dutch cocoa powder content of the mentioned bars.

The values of the available carbohydrate content in the samples were found in the range of 15.54–23.52 g and varied significantly ($p < 0.05$) among products. The

highest carbohydrate content (23.52 ± 0.552 g) was recorded in the case of *Bar 1*, which featured the lowest fibre content (15.97 ± 1.01 g) too.

The fibre content varied between 15.53 and 23.52 g, which is a relatively large interval that could meet to some extent the optimal fibre intake of adult people, which is largely dependent on cultural and economic considerations. However, in order to increase the suitability of PPF bars for elderly people, we would increase the dietary fibre content of such bars, and attempts will be made to accommodate the psyllium husk and the resistant maltodextrin. The resistant maltodextrin (*Astina & Sapwarobol*, 2019) and psyllium husk (*Bacha et al.*, 2022; *Chen et* al., 2022) have known beneficial implications for adults' health conditions.

The PPF-bar-related protein content and total caloric value seemed to look relatively constant, as there were no significant differences detected between the samples. Despite the roughly 27 g of protein that is delivered by a PPF bar, the amino acid and especially the essential versus non-essential amino acid content and their bioavailability or bioaccessibility remain an open question. Moreover, it would be important to consider the use of whey protein isolate to further improve the bioavailability of protein sources in the case of future PPF bars since the amino acid blood concentration following whey protein ingestion was higher than pea and cricket proteins (*Lanng et al.,* 2023).

Total phenolic and flavonoid content

The phenolic and flavonoid content were determined as described in the *Materials and methods* section. The TPC and TFC data were significantly different ($p < 0.05$) among the analysed PPF bars (see *Table 4*).

Table 4. The total polyphenol and flavonoid content of the PPF bars

Bar symbol	Polyphenols (mg GAE/100g)	Flavonoids (mg CE/100g)
	Mean + St. Dev.	*Mean + St. Dev.*
Bar 1	86.39 ± 0.60 ^{b}	85.43 ± 5.11 ^{a}
Bar 2	103.04 ± 3.58 ^{a}	78.85 ± 3.80 $^{a\,b\,c}$
Bar 3	82.36 ± 1.63 ^{b}	75.58 ± 1.20 $^{b\,c}$
Bar 4	90.14 ± 5.80 ^{b}	82.28 ± 0.39 $^{a\,b}$
Bar 5	66.89 ± 1.00 ^{c}	67.46 ± 3.79 $^{c\,d}$
Bar 6	81.93 ± 1.79 ^{b}	54.35 ± 0.59 ^{d}
Bar 7	88.02 ± 2.56 ^{b}	87.09 ± 5.36 ^{a}
Bar 8	73.02 ± 0.97 ^{c}	57.00 ± 3.67 ^{d}

Notes: * Values are expressed as mean ± SD. For each column, values that contain different letters in superscripts were significantly different ($P \leq 0.05$).

Interestingly, among the PPF bars, the highest TPC values were featured by *Bar 2* (103.04 ± 3.58 mg GAE/100g) and *Bar 4* (90.14 ± 5.80 mg GAE/100g), while the lowest polyphenol content was found in *Bar 5* (66.89 ± 1mg GAE/100g) and *Bar 8* (73.02 ± 0.97 mg GAE/100g). The higher content of TPC in PPF, such as in *Bar 2*, where cacao powder was added, is reasonable since the cacao-specific increased phenolic content is known (*Del Rio et al.*, 2011; *Urbańska* & *Kowalska*, 2019). However, the increased TPC in *Bar 4* looks a bit puzzling, but most likely it is due to the differences that might exist among the used protein mixtures.

The highest content of TFC was found in sample *Bar 7* (87.09 ± 5.36 mg CE/100g) followed by *Bar 1* (85.43 ± 5.11 mg CE/100g). The lowest content of TFC was found in sample *Bar 6* (54.346 ± 0.595 mg CE/100g) and *Bar 8* (57.00 ± 3.67 mg CE/100g). The higher content of TFC in sample *Bar 7* is somehow puzzling because if it was due to the strawberry, then we should have seen an increased TFC also in case of *Bar 3.* Nevertheless, the excelling TFC value in *Bar 1* could be potentially attributed to the presence of the banana, whose phytonutrient and flavonoid content was extensively studied (*Mondal et al.*, 2021). Another interesting feature of the TFC is somehow related to the Dutch cacao powder containing *Bar 2* and *Bar 6*, where the differences in TFC values could be put in the context of the different protein mixtures seen in these two PPF bars. These observations regarding the TPC and TFC of the PPF bars and other peculiarities would suggest the relevance of protein mixtures that might confer some additional properties related to the efficiency of polyphenol and flavonoid extractions and bioavailability.

The texture analysis

The analysis of the structure and texture are of great importance because it can offer important information about the chewability of the PPF bars that could affect not just the breakdown pattern but also the digestibility and ultimately bioavailability of nutrients. Undoubtedly, the mastication of any foodstuff and swallowing mechanisms have been shown to adapt and change during the lifetime of humans (*Cichero*, 2017). It has also been demonstrated that the hardness of food affects mastication, and the mandibular movement increases gradually as the food gets harder (*Komino & Shiga*, 2017). Similarly, physiological deterioration like aging, poor dental status, and reduced tongue pressure would result in an affected masticatory performance (MP) that makes chewing more difficult (*Park et al.*, 2022). The MP is also referred to as chewing performance and is the ability of an individual's masticatory system to reduce food to small particles to be swallowed. Experiments have demonstrated that the texture of food would affect the MP, so the number of chews and chewing time increases with the hardness of food though in the case of older people, the MP appears significantly influenced by the nature of dentures (complete versus natural), and the older people with natural teeth exhibit

higher tongue pressure than those with complete dentures. All these observations suggest that the analyses of food texture by assessing the average hardness and maximum cutting forces can offer valuable data concerning the suitability of the developed PPF bars with respect to a specific consumer niche. The analysis of the average hardness and maximum cutting forces were performed as described in the *Materials and methods* section. The obtained results are shown in *Table 5*.

Table 5. The hardness and cutting forces of the PPF bars

Sample	Used protein mix	Hardness force (N)	Cutting resistance force (N)
		Mean + St. Dev.	Mean + St. Dev.
Bar 1	Mix1	43.64 ± 6.55 ^{a}	34.39 ± 0.74 ^{a}
Bar 2	Mix1	7.87 ± 0.78 $^{b\,c}$	11.43 ± 0.48 $^{c\,d}$
Bar 3	Mix1	10.47 ± 2.09 $^{b\,c}$	16.24 ± 0.50 ^{b}
Bar 4	Mix1	8.094 ± 0.08 $^{b\,c}$	11.34 ± 0.33 ^{d}
Bar 5	Mix2	12.51 ± 0.98 ^{b}	13.47 ± 0.53 ^{c}
Bar 6	Mix2	6.43 ± 0.21 $^{b\,c}$	16.16 ± 1.53 ^{b}
Bar 7	Mix2	7.84 ± 1.21 $^{b\,c}$	10.06 ± 0.53 ^{d}
Bar 8	Mix2	4.13 ± 0.66 ^{c}	11.34 ± 0.33 ^{e}

Notes: * Values are expressed as mean ± SD. For each column, values that contain different letters in superscripts were significantly different ($P \leq 0.05$).

Typically, the hardness of high-protein bars is notably increased and tends to rise with the introduction of additional protein (*Li et al.*, 2008). The highest value of the hardness was found for *Bar 1* (43.64 ± 6.55 N) followed by *Bar 5* (12.51 ± 0.98 N), while the lowest hardness value was seen for *Bar 8* (4.13 ± 0.66 N). Noticeably, other samples, such as *Bar 6*, *Bar 7*, and *Bar 2*, were scoring lower values but nearer to *Bar 8*, while *Bar 3* was much closer to the increased value of *Bar 5*.

The observed variances in the hardness of the PPF bars could be related to the differences in the constitutive ingredients. The elevated hardness of sample *Bar 1* could be only partly due to the use of protein mixture 1 (pea protein and rice protein) because this protein mix being used in the formulations of *Bar 2*, *Bar 3*, and *Bar 4* does not result in exceedingly increased hardness values. It is also possible that the banana powder used for the formulation of *Bar 1* confers an elevated rigidity to the texture of this PPF bar. Conversely, the lowest hardness force observed for sample *Bar 8* might be due to protein mixture 2 (pea, rice, hemp, and sunflower), which can result in a more diverse and flexible protein network with a more tender texture (*Zahari et al.*, 2021).

With respect to the protein implication in food texture formation, it has been shown that the different amino acid profiles could influence the formation of the

protein networks (*Małecki et al.*, 2020). Conversely, the lowest hardness force observed for sample *Bar 8* might be due to protein mixture 2 (pea, rice, hemp, and sunflower), which can result in a more diverse and flexible protein network with a more tender texture. For example, lysine and arginine are positively charged at neutral pH, which means they can form strong hydrogen bonds with other amino acids that are negatively charged. These hydrogen bonds can contribute to the formation of a rigid protein network and a harder texture *(Lu et al.*, 2019). Cysteine and methionine are amino acids that are relatively abundant in rice protein extract and can form disulphide bonds with other cysteine residues. Disulphide bonds are strong and can stabilize the protein network. However, too many disulphide bonds can also make the protein bars adopt a hard texture (*Lu et al.*, 2019). Additionally, the functional properties of the proteins can also affect their ability for network formation. For example, pea protein isolate is known to have a good gel-forming property, which stabilizes the protein network and confers a harder texture. Rice protein isolate, on the other hand, is also known to have good emulsifying property, which can make further ingredients bind together but may not contribute as much to the hardness of a given protein bar (*Phongthai et al.*, 2017).

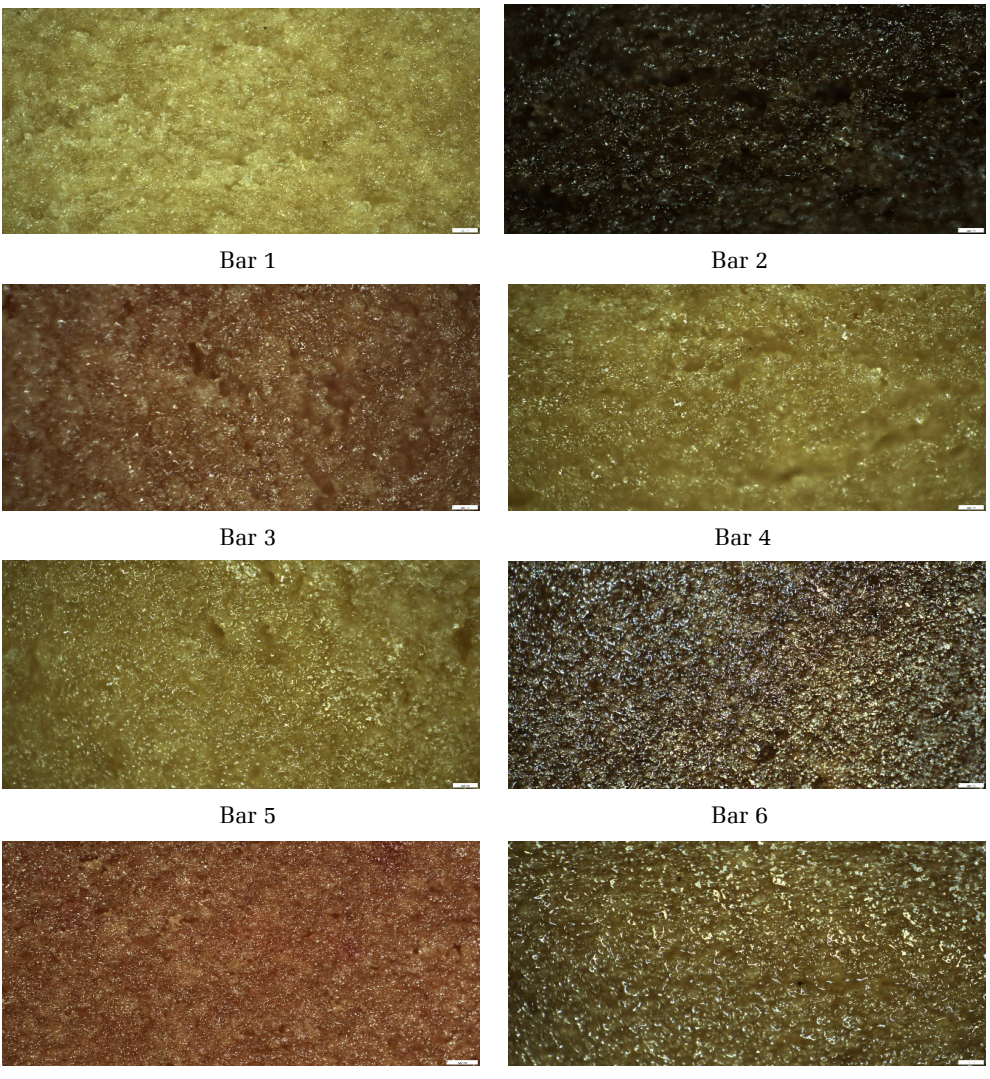
Regarding the maximum cutting force analysis, the results would indicate that *Bar 1* showed an exceedingly high cutting resistance force (34.39 ± 0.74 N), followed by the mostly halved values of *Bar 6* (16.16 ± 1.53 N) and *Bar 3* (16.24 ± 0.50 N) (see *Table 5*).

On the other hand, we found that *Bar 7* had the lowest cutting resistance (11.34 ± 0.33 N) and was closely followed by *Bar 8* (11.34 ± 0.33 N) and *Bar 2* (11.43 ± 0.48 N).

The reduced cutting force observed for *Bar 7*, *Bar 8*, and *Bar 5* might be correlated with the presence of protein mixture 2 in the PPF bars. It seems possible that the use of soluble corn fibre, vegetable glycerin, and chicory root fibre may contribute to a softer texture by increasing the moisture content and reducing the density of the protein network. The above-mentioned ingredients are known to have humectant properties, which means that they can absorb and retain moisture, leading to a softer and tenderer texture (*Małecki et al.*, 2022). Secondly, this is because sunflower oil and lecithin are known to have lubricating and emulsifying properties, which can affect the protein network's ability to form a cohesive structure (*Wang et al.*, 2018).

Furthermore, some of the used flavours may also affect the texture of the PPF bars – for instance, a flavour with a high moisture content, such as fruit, may add moisture to the bar and make it softer. Conversely, a flavour with a dry texture, such as coconut powder, may make the bar harder and drier (*Mazumder et al.*, 2021). In addition, some flavours may contain ingredients that can further modify the texture. For example, a flavour that contains chunks of nuts or fruit may create a chewy texture in the bar. On the other hand, a flavour that contains a liquid or oil-based ingredient may make the bar softer or moister *(Mazumder et al.*, 2021).

Next to the hardness and cutting resistance analysis, we further assessed the texture outlook by monitoring the microscopic cross-section images of the PPF bars (see *Fig. 1*). Food texture is an important feature of our sense and is meant to describe the foodstuff-generated feeling in the month.



Notes: * The images were taken by Microscope model SZX2-ILLK (Japan); the scale bars represent 200 μm.

Figure 1. Microscopic cross-section of the PPF bars

The micrographic image of the PPF bars denotes a fairly uniform chewy type of texture with an even distribution of the particles, and no crunchy outlook can be inferred. It should be noticed that the texture of *Bar 1* appears with more unevenly distributed particles than all the other bars.

Taken together, the obtained hardness- and cutting-resistance-related results correlate with each other (see *Fig. 2*) and also corroborate the micrographic data of the PPF bars.

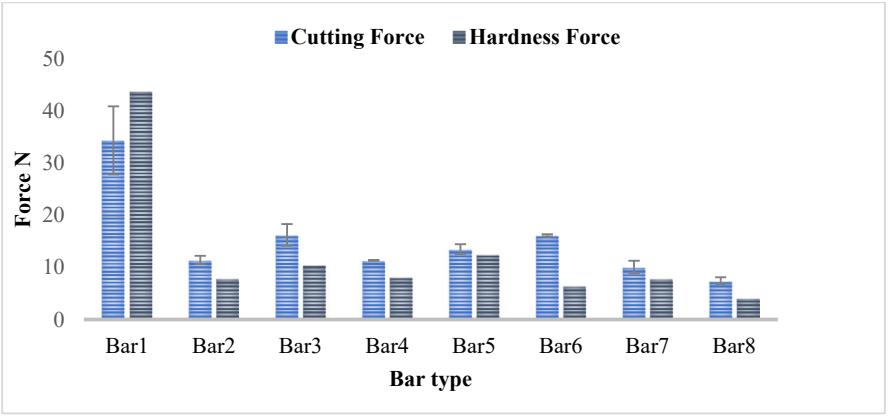


Figure 2. Comparative analysis of cutting and hardness forces data of PPF bars

It is also very important to pinpoint that with the exception of *Bar 1,* all the other, *PPF2–8,* bars are expected not to interfere unfavourably with the mastication performance, and therefore they should score appreciative sensorial grades.

The sensorial evaluation

In order to gain some feedback from customers, we carried out a sensorial evaluation, assessing the appearance, colour, flavour, aroma, texture, and overall acceptability of the PPF bars. The applied method is described in the *Materials and methods* section, while the obtained data are presented in *Fig. 3*. The customer attitudes towards the PPF bars were mostly critical, and they showed great interest in the assessment.

All developed bars were considered acceptable to the participants in terms of appearance. It could be observed that *Bar 6* had the highest scores in terms of appearance, flavour, and overall acceptability, and it noticeably contained protein mixture 2 and the Dutch cacao powder. Also, *Bar 3* was the most preferred by the panellists in terms of flavour, aroma, colour, and texture with mean scores above 7 for most factors. *Bar 3* contains protein mixture 1 and freeze-dried strawberries.

Moreover, *Bar 3* and *Bar 6* scored reduced hardness and cutting resistance force values and seemed to be well-liked by all participants. On the other hand, *Bar 1* had significantly the lowest scores in colour, flavour, aroma, texture, and overall acceptability, which would suggest that this PPF bar has been less palatable or enjoyable to the participants. The low scores of *Bar 1* could be related to its stickiness, less smoothness, and harder consistency, which are features that could be associated with the elevated hardness and cutting resistance values.

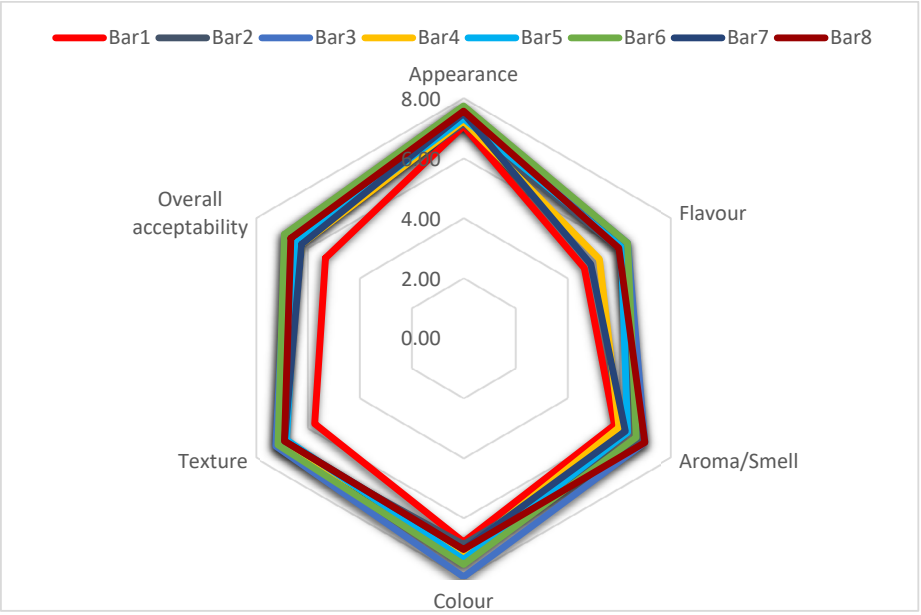


Figure 3. Radar chart of the sensory evaluation of the PPF bars

The sensorial evaluation clearly showed that the overall appearance and texture structure affected the attractiveness and acceptability of the PPF bars. All these observations suggest that some PPF bars, such as *Bar 6* and *Bar 3* containing different protein mixtures and flavours, are suitable for future improvements.

4. Conclusions

Data of the conducted research suggest the influence of the type of protein and food additives used on the textural parameters, nutritional values, and physicochemical parameters. Results showed that the highest polyphenol content values were featured by bars where cacao powder was used, while the highest

flavonoid content was found in the bars where cacao powder and banana powder were added. On the other hand, the parameters of texture profile analysis (TPA) and the cutting forces of the developed bars showed significant differences and could be related to the differences in the constitutive ingredients; the data revealed that adding the banana powder to the formula of the bars would lead to a higher value regarding hardness and cutting force. The sensorial evaluation also revealed that adding the freeze-dried strawberries and Dutch cacao powder did enhance the flavour of the bars. Taken together, the developed PPF bars represent a successful beginning in our quest to increase their suitability and health-promoting effects for senior citizens by paying attention to nutrients such as proteins, polyphenols, and dietary fibres.

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Laboratory-scale extraction of *Hildegardia barteri* (Mast.) Kosterm. seed oil with different solvents, purified with membrane filtration

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Abstract. The shortcomings encountered from the use of conventional methods of refining draw considerable attention when it comes to the processing of edible oils. The present invention to this effect is the usage of membrane technology, which stands out as a suitable alternative, as it offers significant advantages such as minimal chemical additions, low energy consumption, and the retention of nutrients and other desired components. This paper seeks to ascertain the quality of edible oil extracted by different solvents, refined via membrane filtration. Amounts of 25 mL of crude oil sample were extracted with three different solvents (n-hexane, ethanol, and n-hexane-ethanol blend) from seeds of *Hildegardia barteri* (Mast.) Kosterm. and were refined using a micro-filter of pore size of 0.45 µm and an ultrafiltration membrane with 50 kDa cut off before bleaching and deodorizing to obtain table oil. Proximate composition and elemental analyses were carried out on the table oil samples produced and compared with food-grade standards. Results obtained showed 0.133–0.53% moisture content, 0.04% ash, 98.90–99.67% ether extract, 0.23% carbohydrate, and elemental compositions of 51.60–55.00% C, 6.12–6.30% H, 6.21–6.28% O, 0.01–0.02% N, and 13.0–15.0% P for edible oil samples. The findings of this study indicate that edible oil produced from the seeds of *H. barteri* via membrane technology yields good-quality oil for commercial production, except for the need to enhance further reduction of phosphorus content.

Keywords and phrases: *Hildegardia barteri*, edible oils, elemental analysis, solvent extraction, membrane filtration

1. Introduction

The processing of edible oils, which begins with the extraction process, has drawn considerable attention. It has long been a standard practice to extract such oils with an organic solvent to obtain miscella, which is subsequently removed, leaving a crude oil composition. Hexane is commonly used to extract oil because of its lower boiling point and easy separation after extraction and its non-polarity (high oil solubility). On the other hand, hexane is designated by the US Environmental Protection Agency as a hazardous air pollutant (HAP) and has been added to the list of other toxic chemicals (*NIOSA*, 2007). This problem has grabbed the curiosity of scientists seeking an alternative to solvents. Several solvents and mixtures, including n-hexane, petroleum ether, and alcohols, have been tested to obtain oil from *H. barteri* oilseeds (*Aremu et al.*, 2015; *Ochigbo & Paiko*, 2011), and their interfering effects on the nutritional value of the edible oil need to be investigated.

Secondly, after extraction, impurities, such as phospholipids, waxes, organic sulphur compounds, dye compounds, and other contaminants, can make up to 10% of the crude oils obtained. To remove these unwanted compounds, a refining process is needed to make the product useful and marketable.

Membrane technology has been observed to offer remarkable advantages – features include operation at room temperature, minimal chemical additions, low energy consumption, and the retention of all nutrients and other desired components in the oil (*Cheryan*, 1998). These advantages make the separation process stand out as a suitable alternative to the conventional refining method for the food, chemical, biotechnological, and pharmaceutical industries, as it is also known for increased final product quality and separation efficiency (*Fatima et al.*, 2021). Present inventions that relate to one of the methods for refining domestic edible oils is the usage of membrane technology for separation (*Lam et al.*, 2016; *Piacentini et al.*, 2014). However, it has been discovered that various types of membranes and make-up materials have varying degrees of success and inherent constraints. The characteristics of the membrane used impede the complete removal of phospholipids from unrefined oil, needing several different processing stages, such as degumming, refining, bleaching, and deodorizing, to get rid of free fatty acids, phosphatides, particulates, chlorine materials, and the like (*Vaisali et al.*, 2015). One of the goals of these inventions and a significant benefit would be the development of a process for purifying edible oils, wherein most of the hydrous and non-hydrated phospholipids, most of the colour compounds and free fatty acids are separated in the retentate, while neutral oils and the n-hexane pass through the membrane (*Ramos-Andrés et al.*, 2019).

Inferences from different studies carried out suggest that the membrane system technology has the potential for the processing (degumming and deacidification) of fatty oils because it is simple in operation, eco-friendly, and energy-efficient

(*Desai et al.*, 2002; *Chew & Nyam*, 2020). Therefore, this paper seeks to ascertain the quality of edible oil produced from seeds of *H. barteri* by two solvents (n-hexane and ethanol) and their mixture, refined via membrane filtration.

2. Materials and methods

Extraction of *H. barteri* crude oil samples

Crude oil samples were extracted from the seeds of *H. barteri* with three different solvents: n-hexane, ethanol, and hexane-ethanol blend, using a Soxhlet apparatus, and an amount of 25 mL of each sample was collected for further refining.

Refining by membrane filtration

The membrane filtration procedures for the production of edible oil, described by *Van Reis and Zydney* (2007) were employed. The initial step involved pre-filtering crude oil samples using syringe micro-filter with a pore size of 0.45 µm (Millipore Millex-HN, Merck), facilitated by a simple vacuum pump. Subsequently, an ultra-filtration membrane with a 50 kDa molecular weight cut-off and a 15 mL capacity, contained within a centrifuge tube, were utilized to filter the oil samples.

The filtration process was carried out using a fixed-angle rotor centrifuge operating at 5,000 rpm with a duration of 20 minutes. Following this, the filtered oil samples underwent bleaching and deodorization.

Bleaching

The neutralized oil obtained was transferred into a beaker and heated to 90°C. One percent of activated clay (by weight of oil) was added to absorb colouring components. The mix was agitated continuously for half an hour. An increase in temperature to 110°C was allowed for another half an hour. The oil content was then filtrated at the temperature of 70°C by employing a Büchner funnel, filter paper, and vacuum, according to the AOAC technique (*Nkpa et al.*, 1989).

Deodorization

Deodorization was carried out using a modified procedure (*Zulkurnain et al.*, 2013). A rotavapour (Büchi R-210) was used for vacuum distillation process (at $T_{bath} = 95$°C, $p = 4$ mbar), but for a longer period of $t \geq 4$ hours, to get rid of volatile compounds that would cause off-odours and tastes of the oil.

Proximate and elemental properties

The proximate and elemental analysis of table oil samples was analytically determined by using standard laboratory methods (*Helrich*, 2006; *Latimer*, 2016) in three replicates. The oil samples were evaluated for moisture, ash, and crude protein content.

Proximate composition

Determination of moisture content (MC)

Moisture content in the samples was evaluated in line with *Helrich*'s (2006) procedure by dehydrating to constant weight in an oven. The MC was determined by oven dehydration method at 105 ± 2°C till a constant weight.

Determination of ash

The ash proportion in the samples was estimated by following the outlined procedures of *Helrich*'s (2006) method using a muffle furnace at 600°C.

Determination of crude protein

The Kjeldahl method was the procedure adopted in evaluating crude protein in the samples (*Helrich*, 2006). The total Kjeldahl nitrogen content (N_K%) and thereafter the percentage crude protein content (P_C%) of the sample was calculated using the following formulae, (1) and (2):

$$N_K\% = 100 \cdot \frac{(S - B) \cdot N_{HCl} \cdot E_N \cdot D}{m_s \cdot V}, \tag{1}$$

where: S – sample titration reading, mL; B – blank titration reading, mL; N_{HCl} – normality of hydrochloric acid, N; E_N – equivalent weight of nitrogen, with value of E_N = 0.014 mEq; D – dilution of the sample after digestion; m_S – weight of sample, g; V – volume of distillate, mL; and

$$P_C\% = k \cdot N_K\%, \tag{2}$$

where k is the conversion factor of Kjeldahl nitrogen to crude protein, in this case: k = 6.25.

Elemental analysis

The elements such as Carbon (C), Hydrogen (H), Oxygen (O), Phosphorus (P), and Nitrogen (N) of the oil samples were assessed following the methods of ASTM 3174-76 (*AOCS,* 1993).

Determination of carbon, hydrogen, and oxygen

This was determined by weighing 2 g of each oil sample and placing it in a Liebig fragile chamber with sodium hydroxide and magnesium perchlorate. The contents are burned to obtain carbon dioxide and water. The CO_2 is absorbed by sodium hydroxide, while the water is absorbed by magnesium perchlorate. The total amount of *C*(%) and *H*(%) were calculated using the formulae (3) and (4).

$$C(\%) = 100 \cdot \frac{0.2727 \cdot m_{CO_2}}{m_S} \tag{3}$$

$$H(\%) = 100 \cdot \frac{0.117 \cdot m_{H_2O}}{m_S} \tag{4}$$

The mass of the formed carbon dioxide (m_{CO_2}) is determined by calculating the mass increase of the absorbent material ($\Delta m_{a,c}$), i.e. sodium hydroxide ($m^0_{a,c}$), after absorbing the carbon dioxide produced during combustion ($m_{a,c}$), as shown in formula (5):

$$m_{CO_2} = \Delta m_{a,c} = m_{a,c} - m^0_{a,c} \tag{5}$$

The mass of the produced water (m_{H_2O}) is determined by measuring the weight increase of the absorbent material ($\Delta m_{a,w}$), i.e. magnesium perchlorate ($m^0_{a,w}$), after absorbing the water vapour produced ($m_{a,w}$) during combustion, as shown in formula (6):

$$m_{H_2O} = \Delta m_{a,w} = m_{a,w} - m^0_{a,w} \tag{6}$$

Determination of the nitrogen content

Kjeldahl's procedure was used again in assessing nitrogen content in the oil samples, as described for the determination of crude protein (equation (1)).

Determining phosphorus – Tri-acid digestion

The digestion is made using a mix of HNO_3:H_2SO_4:$HClO_4$ in the proportion of 9:4:1. The detailed procedure is as follows: 1 mL of the sample was added to 9 mL HNO_3 and heated for 1 hour at 150°C, and then the solution was allowed to cool. Then, 4 mL H_2SO_4 and 1 mL $HClO_4$ were added, the mixture was heated for 1 hour at 150°C, and the solution was cooled. Deionized water was added to the final volume of 100 mL (*Tandon*, 2001).

Estimation of the phosphorous content of the sample digest was obtained by transferring the sample to a volumetric flask:

$$P(\%) = C \cdot \frac{1}{m_S} \cdot \frac{100}{m_{alq}} \cdot \frac{V_f}{1000}, \tag{7}$$

where C – concentration of (P) in the digested sample, mg/L; m_S – sample weight, g; m_{alq} – aliquot weight, g; V_f – final volume, mL.

Colour analysis

The colour analysis of oil samples was performed using the Color Tec-PCM colorimeter (Color Tec, Clinton, NJ). The analysis is based on the CIE L*, a*, b* (CIELab) colour scheme established by the *Commission Internationale de l'Eclairage* (*CIE*, 2020). The measurements obtained are displayed in CIELab format, as described in *Belbin* (1993). The L^* value represents the lightness-darkness dimension, the a^* value represents the red-green dimension, and the b^* value represents the yellow-blue dimension. The hue of the colour (h°) is calculated as:

$$h^\circ = \arctan\left(\frac{a^*}{b^*}\right), \tag{8}$$

where $h^\circ = 0^\circ$ for red, and $h^\circ = 90^\circ$ for yellow.

Additionally, chromaticity (C^*) is calculated by formula (9):

$$C^* = \sqrt{a^{*2} + b^{*2}} \tag{9}$$

Statistical analysis

The significant differences among oil parameters were evaluated using one-way ANOVA (*SAS*, 2002) and laid in a completely randomized design, which was run on SAS statistical software.

3. Results and discussion

Proximate composition

The values of proximate composition obtained in *Table 1* reflect the nutritional value of a refined food-grade level of *H. barteri* oil samples that could be recommended for consumption.

Table 1. Proximate composition of the oil samples extracted with different solvents

Component	Extraction solvent			p-value
	n-Hexane	Blend	Ethanol	
	m/m%			
Moisture	0.53 ± 0.06^{a}	0.53^{a} ± 0.21^{a}	0.13 ± 0.06^{b}	0.014
Protein	0.20 ± 0.10^{a}	0.10 ± 0.10^{ab}	0.00 ± 0.00^{b}	0.064
Ash	0.04 ± 0.06^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.000
Ether extract	98.90 ± 0.10^{b}	99.37 ± 0.3^{a}	99.67^{a} ± 0.21^{ab}	0.015
Carbohydrate	0.23 ± 0.058^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.000

Notes: Blend: n-hexane:ethanol 1:1 (V/V); means ± SD of triplicate values with similar notations are not significantly different; significant at $p < 0.05$.

The MC obtained for all oil samples ranges between 0.13 and 0.53%, as the ethanol oil extract had the lowest value (0.13%) and was significantly different from both hexane-extracted (0.53%) and blend-extracted (0.53%) oils. The obtained result was much lower than the results reported from other studies such as for *Coco nucifera* oil: MC = 8.43% (*Evbuomwan & Emmanuel*, 2019) or *Moringa oleifera* seed oil: MC = 10.5% (*Adegbe et al.*, 2016). However, values were higher than the maximum limit recommended by the ASTM standard (0.05%). Consequently, having low MC will allow for the longer shelf life and stability of the products, as they will not be easily susceptible to rancidity.

The protein content obtained in all food-grade oil samples ranged between 0.1 and 0.2% and was low and insignificant. No noteworthy differences were observed

in the protein content among the samples. The low values obtained show loss in protein content as compared to the range (1.29–1.66%) obtained from the different extracted crude oil samples of *H. barteri* (*Adeniyi & Oluwadare*, 2016) and serve as a proof that protein denatures as it is exposed to higher temperatures during the process of refining.

Ether extracts, which are synonymous to crude fat, ranged between 98.90 and 99.67% for all edible oils produced, and the statistical analysis indicated no significant difference among them. The result indicates not less than 98% pure oil content and ≤ 2% of other components, which could be attributed to impurities incurred during handling or processing, and this also correlates and affirms the result obtained for ash content in the oil samples.

Results for ash content and carbohydrate show that only the hexane oil sample was observed to have a low proportion of 0.04% and 0.23%, respectively, and it was not detected in other samples. The value of ash content, which indicates the inorganic or mineral content left in a food sample after it has been heated to a very high temperature (*Alinnor & Oze*, 2011), was only detected in the n-hexane-extract sample (0.04%). Though a little higher than the ASTM permissible amount (0.02%), it can be said to be within the range and compares well with the standard. The presence or absence of ash indicates a link to the processing and the interference of the different solvents used in extracting the oil from the seed oil. The absence of ash content in ethanol and hexane-ethanol blend extracts indicates the purity of the samples. The determination of ash content ensures that no toxic minerals are present and hence the safety of the oil sample.

The carbohydrate content obtained in just one of the samples (hexane extract) proves that the inherent energy content of oils is not based on carbohydrates (polymer of simple sugars) but on the fatty acids, which are the carboxylic group of lipids that yields a large quantity of adenosine triphosphate (ATP) when metabolized. This also suggests that there could be little influence of the different solvents used for the extraction of crude oils on the proximate composition.

Elemental analyses

Results in *Table 2* summarize the mean values ± SD of the elements analysed on all table oil samples.

For all oil samples, it was observed that the elements were of low values in all table oil samples except for phosphorus (*Table 2*). This, therefore, suggests that the phospholipid component, which has P as the major element, was not efficiently removed by the membrane-refining method. This was corroborated by *Koris and Vatai* (2002), who reported that the complete removal of phospholipids from the crude oil is impeded by the characteristics of the membrane employed, thus calling for a variety of processing steps.

Table 2. Elemental composition of the oil samples

Element	Extraction solvent			p-value
	n-Hexane	Blend	Ethanol	
	m/m%			
Hydrogen (H)	6.14 ± 0.02[b]	6.30 ± 0.02[a]	6.12 ± 0.02[b]	0.000
Carbon (C)	55.00 ± 0.01	51.60 ± 0.02	54.30 ± 0.02	0.296 (NS)
Oxygen (O)	6.24 ± 0.02[ab]	6.21 ± 0.02[b]	6.28 ± 0.03[a]	0.014
Nitrogen(N)	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.054 (NS)
Phosphorus (P)	15.00 ± 1.00[a]	13.67 ± 0.58[ab]	13.00 ± 1.00[b]	0.079 (NS)
Total	82.39	77.80	79.71	

The nitrogen content (N%) obtained for the set of samples (0.01–0.02%) was not significant as compared to 1.94% obtained in grape seed oil as reported by *Luque-García and Luque de Castro* (2004). This elemental test was carried out to document the quantities of elements obtained after adopting the membrane-refining process and to know the extent of reduction of phospholipids in the seed oil of *H. barteri*. The removal or reduction of phospholipids during refining reduces the final content of phosphorus, which is a pro-oxidant that could accelerate the rancidification of the oils (*Choe & Min*, 2006).

CIELab oil colour

Results shown in *Table 3* reveal significant differences ($p < 0.05$) in L^* a^* b^* values of oil extracted using n-hexane, ethanol, and their blend. The L^* value was highest in ethanol-solvent-extracted oil followed by blend, the lowest being found in n-hexane. The n-hexane-extracted oil colour had the highest a^* value followed by blend and the lowest value in the ethanol-extracted oil. The b^* value was highest in hexane-extracted oil followed by blend, and ethanol-extracted oil had the lowest value.

The Chroma, which provides a quantitative measure of the colour saturation, had a highest value of 83.17 for hexane-extracted oil followed by 37.71 in blend-extracted oil and the lowest in ethanol-extracted oil, with a value 31.83. Colour is an important aesthetic quality parameter, which, according to the definition by the CIE, is the characteristic of visual perception that can be described by the attributes of hue, brightness or lightness, colourfulness, saturation, or chroma (*Fairchild*, 2013).

The *Hildegardia* seed oil has the highest value of yellowness, which suggests the higher concentration of highly lipophilic yellow pigment carotenoids such as carotenes (β-cryptoxanthin, α- and β-carotene) or xanthophylls with chemo-

preventive efficacy in animal models of skin carcinogenesis and buccal pouch carcinogenesis (*Nagao & Yanagita*, 2008). The higher value of b^* may be due to the higher solvation power of hexane in extracting carotenoids, of blend-extracted table oil, followed by a lower value of $b^* = 37.57$ with ethanol-extracted oil as the least significant $b^* = 31.83$; this may be due either to the lower solvency power of ethanol to extract carotenoid (*Ordóñez-Santos et al.*, 2017) or to its superior solvation power to extract chlorophyll, which is obvious in its negative colour coordinate $a^* = -0.41$, a result tending towards greenness, denoting the presence of chlorophyll, a green pigment, and also a lower $a^* = 3.2$ in 50% blend-ethanol-hexane-extracted oil, while hexane-extracted oil has a significantly higher $a^* = 10.25$; the psychometric indices of lightness for oil in this study varied significantly (*Figure 1*).

Table 3. CIELab colour analysis of the extracted oil samples

Colour parameters	**Extraction solvent**			**p-value**
	n-Hexane	**Blend**	**Ethanol**	
L^*	78.38 ± 0.34^b	81.88 ± 0.83^a	82.90 ± 0.49^a	0.0002
a^*	10.25 ± 0.46^a	3.20 ± 1.17^b	-0.41 ± 1.25^c	0.0001
b^*	82.54 ± 0.23^a	37.57 ± 1.57^b	31.83 ± 1.53^c	0.0001
Chroma	83.17	37.71	31.83	-
Hue ($h°$)	83.82	87.50	87.39	-
Chromaticity	Yellow-Red	Yellow-Red	Yellow-Reds	-

Figure 1. Oil samples extracted by different solvents

Lightness of blend- (81.88) and ethanol- (82.90) extracted oil compares well with the fresh variety of virgin olive oil L^* = 82.97, as reported in the literature (*Ceballos et al.*, 2003). Often, lighter colour has been associated with better-quality oils, especially for salad oils and shortenings (*Shahidi*, 2005).

4. Conclusions

According to the outcomes obtained from this study, the use of membrane technology and further refining applied for the production of edible oils from the seeds of *H. barteri* yielded lower moisture content in the oil samples. Consequently, a longer shelf life, stability of the products, and non-susceptibility to rancidity are attained. The solvents did not affect the ash content of the extracted oil, which was very low or undetectable. Different solvents used for oil extraction impact colour attributes, including lightness, yellowness, greenness, and colour saturation. The lightness values varied significantly, but both the blend- and ethanol-extracted oils had lightness comparable to high-quality virgin olive oil. Hexane extraction yielded a higher b^* value, indicating the greater concentration of yellow pigments, which could possibly be carotenoids. The n-hexane's superior solvating power for carotenoids could explain this outcome.

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